GROWTH POTENTIALS OF THE SKELETAL DEVELOPMENT WITH SPECIAL REFERENCE TO THE CRANIOFACIAL COMPLEX DURING POSTNATAL DEVELOPMENT IN THE RAT

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"I suppose that every writer hopes that his contribution is worthwhile and will lead other researchers into new and profitable fields. I feel strongly, though perhaps naively, that it is very important to understand the various ways in which mammals respond to environmental situations in the hope that we may see indications of how another mammal, the human species, could respond. The human race is in the process of rapidly throttling itself by its reproductive prowess and it is possible that, despite man's belief that he can control his own environment, the environment he "created" is catching up to the extent that it may soon be drastically affecting his ability to reproduce. If this does happen, and if man does respond in a basically mammalian manner, it is to be hoped that the responses of other mammals may indicate to us the types of reproductive phenomena we may expect to see develop in the human species."

SADLEIR 1969

DECLARATION

I hereby declare and affirm that the Thesis entitled Growth Potentials of the Skeletal Development with Special Reference to the Craniofacial Complex during Postnatal Development in the Rat is entirely my own work and composition.

A. Wallace Park

May 1975

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Turning to the world of the visual, the expertise of Miss M. Benstead has once more graced my work - along with other works more profound - and some say she hath a greater knowledge of rats than I! Such a comment I find difficult to dispute.

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SUMMARY AND CONCLUSIONS

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The concept unifying the present work stems from Gestalt theory, its involvement in the holistic approach, and the fact that the growth of the rat is more than an aggregate of its parts. The growth of the rat remains within a "central" control with regions such as the craniofacial complex retaining certain specific patterns of growth. The interrelationships of the whole (body growth) and part (craniofacial growth) have been examined to prevent an "isolated" study of a part being undertaken without perspective being achieved. The factors are diverse and variable and therefore require insight to be gained from the growth pattern of the whole before establishing the specific pattern of growth of the part. Volume I contains various studies of the growth as a whole which thereby forms a significant basis for the remaining work dealing with the complexities of the craniofacial region.

A) Experiment I. The Influence of Numbers

Uniformity of growth of laboratory animals is of direct interest especially since the causes of variation depend on the genetic endowment, environmental factors with persistent effects, and environmental factors with transient effects. In the environmental field, maternal effects introduce important changes in the growth potential of young suckling rats as demonstrated by weight and length.

The present experiment deals with the "standardisation" of the growth potential of rats during the preweaning period, the variance of which appears to be mainly attributable to the phenotype and experience of the mother. A postulate indicated (FALCONER, 1947) that the 12-day weight of a standardised litter of mice might well reflect the amount of milk available, and it was found later that 60% of variation in individual 12-day weight was due to the maternal influence (EL OKSH et al 1967). Recently it was shown (EISEN et al 1970; EISEN and HANRAHAN, 1970) that much of the maternal variation was due to environmental or non-additive genetic effects.

Any move towards uniformity is an important asset and thus the question arises of what is the best size of litter which an average rat mother can raise which allows the expression of the fullest growth potential. The influence of litter-size was investigated by means of albino rats (Sprague-Dawley strain). The breeding females for the experiment were all "proven" mothers and their litters were arranged so that birth occurred within a few hours of each other. At birth the young were mixed to reduce any potential genetic differences and were distributed by random selection to the mothers - the cross-fostering technique. Litters consisted of rats numbering from 2 to 15 per litter. The first 24 hours after birth was regarded as Day 1, and at this point and thereafter at 24-hourly intervals, the litters were weighed en masse and the mean weight of the single specimen calculated. The results were processed and a linear regression analysis carried out.

Body growth by weight and length during the preweanine phase of development (birth to day 20) is known to be triphasic. This consists of breaks in the regression lines occurring at day 5 and day 15. The first phase (0-5) covers the rising milk supply, the second phase (5-15) covers the peak of milk supply and the gradual waning, while the third phase (15-20) involves the intake of solid food. The litter-sizes in the experiment reflect all these phases and show the extremes of the lactational capacity.

Litters consisting of 2-3 young did not achieve their full potential growth rate as compared to litters of 4-7. Litters ranging from 8-10 did not grow well while litters of larger numbers of 11-15 showed signs of retardation.

From these observations, it appears that the external stimulatory factors of lactation are insufficient from very small litters, while giving a maximum level to the large litters. The increased lactational capacity of the mothers of large litters is nullified by the greater demands and uneven distribution to the young. Those litters containing 5-6 young rats appear by weight to be near or on the point which satisfies the growth potential.

B) Experiment II. Maternal Capacity and Stimulation

In Experiment I the search for variables controlling the development of the young rats established the "standardised" litter of 5-6 young per mother. It is known that there is an inverse correlation between litter-size and maternal behaviour with the underlying lactational influence. There are also suggestions that the natural reduction of the litter-size by infant mortality enables the remaining young to utilise both the extra milk available and their own growth potential.

The present experiment was evolved to examine the maternal capacity in relation to the stimulation of the mother by using large litters followed by reducing the litters at selected periods and noting the growth pattern of the smaller litters so formed. The experimental litters stemmed from 18 female rats selected on their proven breeding and maternal characteristics. The young rats were mixed at birth and re-allocated by the cross-fostering technique to their "mothers" to form litters of 14. Changes to these litters took place on days 5, 10 and 15 - based on the observations of the triphasic growth pattern - and were initiated so that the litters consisted of 4, 6, 8, 10 and 12 young.

Thus on Day 5: Litters coded A to E were reduced as follows:-
A to 4, B to 6, C to 8, D to 20 and E to 12 rats per litter.

Day 10: Litters coded F to K were reduced to:
F to 4, G to 6, H to 8, I to 10, J and K to 12.

Day 15: Litters coded L to R were reduced to:
L and M to 4, N to 6, O to 8, P to 10, Q and R to 12.

A control litter of 14 was designated as S.

Following their formation each litter was weighed at 24-hourly intervals. When the mother was stimulated by a large litter, the lactation increased relative to the size of the litter. Following a short period of stimulation the increased milk supply could be used to benefit the litter, but only if the litter was reduced in numbers. The litter-size making the best use of the milk was found to range between 5 and 7 young. Litters above this number did not show a uniform progress. The timing of the litter reduction was crucial and had to be made on day 5, thus a "standardised" growing rat with ample milk supply for full use of its growth potential is only possible after five days. The size of the stimulatory litter of 14 also appeared to be crucial since this number resulted in some instances in growth retardation occurring before any reduction of the numbers was possible. From the results it is possible to postulate that a stimulatory litter should consist of 8 rats at birth and that to achieve the fullest growth potential, the subsequent

litter-size should consist of 5 or 6 rats per mother.

Details of the physiological and maternal behavioural patterns in relation to lactation and litter-size were discussed in the light of current hypotheses and awareness made of some of the complications arising out of the interactions of these two main controlling factors.

C) Patterns of Preweaning Body Growth

As a basis of skull study, an extensive examination of the patterns of body growth were made. Basically two separately bred samples were examined each being sub-divided into small (6) and large (15) litters. Some 2,000 rats were involved. The small and large litters were formed by the cross-fostering technique at birth. The basis of the large litters was to obtain a reduction of the amount of milk available to the young rats by overtaxing the mother's milk producing capacity. The pattern of body growth was examined by means of weight and head-body length and both showed a characteristic triphasic spectrum which was evident in both small and large litters. Linear regression analysis showed that the First Phase covered the period of birth to day 5, while the Second Phase covered the period of day 5 to day 17 in the head-body length of both samples of small litters. The large litters showed a slight difference of 1 day between the samples, i.e. day 5 to 15 and day 5 to 15. Examination of weight showed the small litters to have phases of 5 to 17 days (sample 1) and 5 to 15 days (sample 2) whereas the large litters showed uniformity in having their Second Phase period covering day 5 to 14 in both samples. The Third Phase starting point depended on where the Second Phase had ended but regression line analysis indicated a different growth rate up to the final day.

The triphasic spectrum stems from the interplay of the external environment and the genetic endowment. Variables implicit in the concept of "external (maternal) environment" have been represented by those of "nutrition". The control exerted by these two factors on the growth have been confirmed by experimentation of litter-size.

D) Patterns of Postweaning Body Growth

The postweaning development of the small and large litters, based on the cross-fostering technique at birth, was examined at 24-hourly intervals over a period of 20 days (days 20 to 40). This period was selected to allow growth alterations due to the reduction of milk supplies, a similar period of recovery time on a solid diet. The characteristic changes of both weight and length were examined by distribution charts and the sexes separated throughout.

The most obvious change, apart from the much slower growth of the large litters compared to the small litters, was the variance within each litter type. It is suggested that this variance stems from the restrictions of the maternal environment being lifted and the full play of the genetic endowment is being allowed to express itself.

Sexual dimorphism was not found to be significant before day 30, thereafter the difference by weight and length became evident. From day 33, the small litter weights pass the 100 g mark but the large litters do not reach this level by the final experimental day (day 40). It is evident that the catch-up period of some 20 days is not the only factor which may be insufficient, there is also the problem of the damage to the growth mechanisms and the timing of the growth potential.

Obviously the growth potential during the preweaning period is extremely susceptible to adverse food conditions. Within every large litter there exists a considerable range of individual weights attained and it appears that the heavier individuals, by size alone, are better suited to reach their characteristic species-size (principle of equifinality) than their smaller, lighter, litter-mates. The conditions induced on the maternal capacity of the large litters ranging from 13 to 18 individuals per litter during the preweaning period have, without doubt, a marked and apparently lasting influence on the growth potential of the rats over the postweaning period even in the presence of unrestricted food supplies. Within the limits of the present work it is obvious that a much greater recovery period will be required to finalise the outcome of a recovery of growth position.

E) Craniofacial Morphology

Skeletal descriptions of the rodent skull were found to be inadequate in the current literature both in visual details and terminology. In order to ensure accuracy the morphological features of skulls of adult rats were recorded with half-tone drawings of various selected views. The drawings represented the features of the skull built from a number of male and female skulls of adult status. A total of 14 different views were obtained to allow perspective to be in full for the external morphological appearances. The individual bones forming the skull were separated with the help of ultrasonic waves. The disarticulated bones were arranged to form an "exploded" pattern and the external and internal features recorded.

The resulting rodent skull atlas is the most complete available and it is hoped to publish it at a later date.

F) Craniofacial Morphology - The Osteodental Fissure

The morphogenesis and regression of the osteodental fissure of both the maxillary and mandibular regions have been examined in relation to tooth eruption in control and experimental litters with the emphasis on the bone.

Fissural formation was found to occur above the developing first and second molars of the maxillary and mandibular regions, while regression of the fissures was found to take place when the cusps of the molars penetrated the overlying alveolar bone behind the leading edge of the crest. The experimental litters with reduced milk supply showed a slower formation of the osteodental fissure gradually building up to a two day deficit. This particular deficit was paralleled by a similar one in the eruption of the molars through the bone, although once having erupted, the difference between the control and experimental was reduced to one day.

Formation of a fissure over the third molar regions did not occur and the teeth were completely encapsulated by bone. Eruption of the third molars took the form of a wedge of disintegrating bone at the mesio-occlusal aspect. This wedge extended distally until the molars were exposed. Third molars were not found to manifest any significant changes in their eruption pattern due to experimental conditions.

G) Craniofacial Morphology - Tooth Mineralisation

The chronological sequence of cusp and crown appearance of the first, second

and third mandibular molars, and in later stages their respective roots, have been observed in relation to their mineralising potential. The tooth material stemmed from control well-fed litters and experimental under-fed litters, and was subjected to X-rays under standard conditions of exposure and processing.

The sequence of cuspal mineralisation of the first molar of the control group shows a difference of 2 to 3 days when matched with its experimental counterpart. The second molar, which under normal conditions begins to develop two days later than the first molar - has a difference of mineralisation of at least 1 day between its control and experimental groups. The third molar mineralisation shows no significant differences between the control and experimental groups. Observations on the root formation of these teeth did not reveal any significant differences.

The differences in the mineralising pattern can be related to the general triphasic growth pattern which exists for parameters of body and skull growth. Those teeth such as the first and second molars which fall within the limits of the first and second phases ... 0-5 days and 5-15, expose their developmental processes to the vagaries of the maternal environment which is responsible for the major portions of the growth support. As the maternal environment declines and the external environment takes over the control of food, teeth such as the third molar are allowed to develop under much more advantageous conditions and those which encourage them to express their inherent growth potential.

As far as the method has allowed, it is clear that during certain phases with nutritional deficits, the formation of the crowns of the first and second molars are retarded in their mineralising, the first molar more than the second. This retardation manifests itself for a few days and finally the teeth assume a more standard appearance. Thus, undernutrition with the maternal environmental field does slow the growing process of the teeth but does not appear to inflict any visual permanent damage since the lost ground is eventually recovered. Those teeth such as the third molar which are subjected to only a little maternal environment and which develop mainly under the mantle of the external environment, do not manifest any mineralisation lags.

In conclusion, the recovery of the first and second mandibular molars from the imposed experimental conditions serves to underline the "Principle of Equifinality" as enunciated by BERTALANFFY (1960).

H) Craniofacial Morphology - Mineral Profile of Whole Skull

A randomly selected sample of control and experimental skulls taken from the total sample of 204 preweaning skulls was examined by means of a Norland-Cameron Bone Mineral Analyzer. The mandibles were not included in the survey. The skulls were positioned on the Scanner Module so that the longitudinal scan (antero-posterior) and a transverse scan (across the zygomatic roots) could be taken. The width of the analysis was within a 5 mm limit and the Computer Module was linked to a recorder to give a profile of the mineral density.

The series consisted of control and experimental skulls ranging from 2 to 19 days old and the tracings derived from both were compared within their specific age group.

Results of the tracings of the older skulls revealed a characteristic profile

for both longitudinal and transverse scans. The longitudinal profile consisted of 6 high mineral density peaks and 5 troughs of low density. The 6 peaks were related to various bone and tooth structures but only 2 of the troughs could be matched to structures with certainty. The transverse profile produced a characteristic outline of 4 high density peaks and 3 troughs of low density. The 4 peaks were related to various structures but the troughs were not classifiable for use.

The longitudinal profile could be classified by its appearance - between day 2 to day 6, since only the anterior and posterior high density peaks could be found; from day 7 to day 13 the peaks of high density numbered 4, while from day 14 to day 19 all high density peaks - 6 in all - were seen. The degree of peak height varied with the age and with the control and experimental skulls. The transverse profile showed all the high density peaks from day 4 upwards. In both the longitudinal and transverse profiles there was considerable variation mainly due to the size of the sample and the variation of the litter-size and individual growth rates.

Comparisons between control and experimental profiles showed that as well as the mineral differences originating from the difference in the skull sizes, there was evidence of a general relative reduction in the mineral content of the experimental skulls.

I) Craniofacial Morphology - Preweaning Skull Growth

The pattern of skull growth during the period 1-20 days reveals a triphasic spectrum based on regression line analysis. All the phases have their own characteristic growth rate which depends on the specific dimension of the skull in use. In general terms the triphasic pattern mirrors that found in the weight and length studies which proves that the growth process must respond to some overall control. Variables implicit in the concept of the external environment are regarded as those of "nutrition" and the use of "large litters" produced a considerable nutritional insult to their members. The growth spectrum within the experimental litters remained triphasic with a few variables.

The intrinsic growth control of the body as a whole has been related to the triphasic growth pattern of body weight and length and to the skull dimensions. Factors controlling growth appear to be varying degrees of autonomous and pituitary system with some genetic endowment. Throughout all the parameters of body and skull, the first phase has remained constant in its period of activity. All the regression coefficient "b" calculations indicated a break of line at day 5. In the body parameters the growth rates in the experimental group were lower than in the control - this being due to a greater sensitivity of weight, etc. to environmental changes. The skull parameters, however, exhibited no environmentally induced changes in the first phase.

Experimental skull parameters showed the effects of undernutrition in the second phase and shared the characteristic of having varying periods, in which the time fluctuated considerably relative to the control second phase, with those of the body second phase. In the skull dimensions the growth rate of the second phase was specific for each dimension. Although the second phase appeared to be under the control of a joint autonomous-pituitary system which maintained the presence of the phase as an entity, the length of each specific second phase depended on the inherent growth potential of each dimension. Thus the presumably "blanket"

effect of the undernutrition only served to highlight the potential or state of a specific dimension. This particular effect is further complicated by the dimensions being formed of single or several bones, those with more than one bone reflected the summation of the member with the stronger potential thus giving a negative or positive result which did not take the weaker potential into account.

By an arbitrary division of the skull, the growth of the facial bones have a slow deceleration while the earlier developing neural bones have a rapid deceleration. In the second phase the undernutrition depressed the growth of all the bones but relative to their specific potentials. Thus the pattern of growth exhibited by the control dimensions in the second phase was repeated in the experimental second phase but with a relative fall in growth rate. Apart from this growth rate difference, the experimental second phase showed an earlier breakage of the regression line in those dimensions which had a slow deceleration while a later line breakage in those with a rapid deceleration. In other words, those dimensions being rapidly decelerated due to their near-completion of normal growth were being depressed even further. The early breakage of the regression line and hence the start of the third phase probably stems from a premature weaning caused by lack of milk and the search and assimilation of solid food. This is a behavioural change which eventually is reflected by the growth-rate change. One variable in the growth pattern was the appearance of several biphasic dimensions and these proved to be very slow growing bones whose figures were difficult to measure. In some instances these biphasic dimensions exhibited a triphasic pattern in the experimental group. The calculations from the regression analysis often indicated a triphasic pattern but the figures were so small that a line breakage could not be constructed.

The third phase of the growth pattern showed a behaviour which could be related to (a) the specific growth potentials of the various dimensions, i.e. slow or fast decelerators, and (b) the alteration of the food source. Those dimensions which had previously been having a slow deceleration followed by a depression in the second phase appeared to have retained considerable growing qualities since their growth rate increased to be several times greater than observed in the control third phase. On the other hand, those with the fast deceleration continued that trend.

Directional growth control of the skull remains within the intrinsic autonomous-pituitary system with a little genetic, while the fluctuations of the growth rest upon the maternal environment and later the external environment. Because the skull has differential growth rates the timing of a nutritional insult means that a number of the bones will not retain the required growth potential to enable them to join a "catch-up" process when food supplies are eventually raised. On the other hand, those bones which do appear to have regained their zest for growing with the new food source, and join the "catch-up" process, may not be able to maintain it. The fact that these bones were undergoing a slow deceleration originally indicates that the time must be limited.

J) Craniofacial Morphology - Postweaning Skull Growth

Patterns of skull growth during the period covering day 21-day 40 reveals aspects of the growth gradients observed in the previous day0-day 20 series. The Composite Division showed its three dimensions - Cranial length, Braincase length and Zygomatic length to be retarded by day 40 in the experimental group.

In the Facial Division, Facial height, Nasal bone width and length, the Least Interorbital width, Facial length, Palatine Bone length, Maxilla-premaxilla length and the Mandibular length (c) all appeared to have reached a characteristic-species final size for day 40 relative to the control dimensions. On the other hand, the Bizygomatic width, the Frontal Bone length, the Palatal length and the Mandibular length (a) were all retarded. The Neural Division consisting of the Braincase width, Biparietal width and Cranial vault length all showed retardation. Finally, the Basal Division formed by the Basispneoid and Basioccipital lengths and the Basioccipital width also showed retardation.

These results reflect the basic growth pattern of the skull during the triphasic stage of development (0-20 days) especially during the second phase. Within the second phase the various skull dimensions indicated a deceleration of the growth rate which differed from dimension to dimension. In the neural group the deceleration was rapid while in the facial group many of the bones showed a slower deceleration. The neural group is regarded as being the result of the brain growth cessation (in terms of cell number and DNA). apart from some bones which increase to support the muscle development. The facial bones in most instances show a fairly vigorous growth and have a greater relative growth rate compared with the neural group.

The bones of the craniofacial complex do not completely cease to grow but continue at a slow linear growth rate throughout the period of the investigation. Those bones with a slow deceleration of growth rate have the greater opportunities of reaching the level attained by the control group. Bones or dimensions which are either adjacent to or fully implicated with a rapid deceleration of growth have a low response to adequate food supplies. There are a number of dimensions which appear to have a slow linear growth over much of the period and these do not exhibit measurable changes useful for comparison between control and experimental groups. In all, there are 13 dimensions out of the total of 22 which show degrees of retardation in the experimental groups. Of these, the neural, basal and composite groups provide 9 while the facial provides 4. Following a reduction of the maternal lactation the growth potential of most of the facial bones remains at a level which allows a loss of growth to be recovered by day 40.

Thus it can be stated that for those facial bones that have recovered their deficits, the principle of equifinality (BERTALANFFY, 1960) appears to be operating but for the remaining skull dimensions and body weight and length, the present series does not indicate that a full recovery is assured. Further to this, there are no means within the series to calculate the presence of degree of tissue damage which may have been inflicted by the size of the experimental litters.

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C O N T E N T S

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PATTERNS OF CRANIOFACIAL MORPHOLOGY

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PART I

INTRODUCTION

As ideas are preserved and communicated by means of words it necessarily follows that we cannot improve the language of any science without at the same time improving the science itself; neither can we, on the other hand, improve a science without improving the language or any nomenclature which belongs to it. However certain facts of any science may be, and however just the ideas we have formed of these facts, we can only communicate false impressions to others while we want words by which these may be properly expressed.

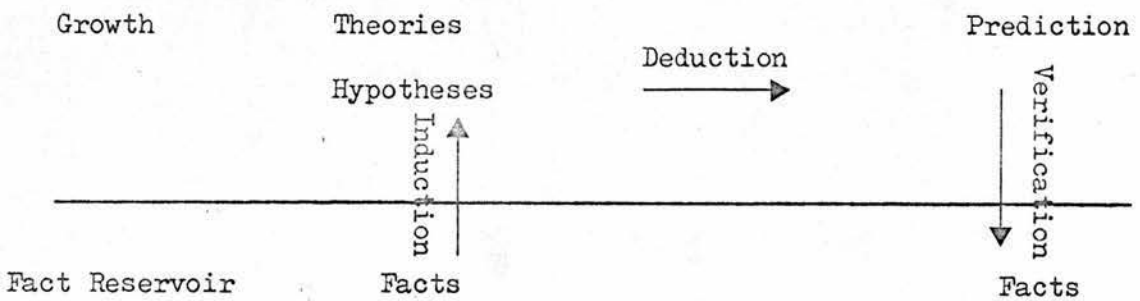
Lavoisier, "Elements of Chemistry", Preface.

1.1 A Priori ..... a Posteriori ?

> "And if you take one from three hundred and sixty-five, what remains ?"
>
> "Three hundred and sixty-four, of course."
>
> Humpty Dumpty looked doubtful. "I'd rather see that done on paper," he said.

The thought of the Greeks isolated and at peace while conceptualising, answers the first, while sallying forth to observe the facts, answers the second.

Stemming from these words is a greater depth of scientific philosophy and practicality, the elucidation of which lies beyond the scope of this thesis. However, between the two extremes of "conceptualisation" and "observation" the most characteristic feature of any science is its method. Science must start with facts, irrespective of what theoretical structures it forms, and end with facts. There is the observation followed by the description and comments of what the future holds. Finally, predictions are made on the basis of the theories formulated which in turn should be compared with the facts. One can then say that the method is cyclic since facts form the initiation and termination. From one such cycle will arise new ideas and a new cycle will commence - giving us an endless chain of progress.



The diagram gives the basic outline of the "method" employed in Science,

the world above the horizontal line mainly belonging to the "theoretical" world while that below covers the "unmined facts" and the "miners".

Let us now look at the "unmined" facts and regard them as having been extracted - one could refer to them as "grains of sand" - and within the scientific endeavours which occur there are many such grains of sand. Some of the grains are of immediate value since some jigsaw of knowledge has a crucial piece missing, but many of the grains lie in scattered perfusion throughout the scientific literature. When this situation is commented on, it is met by the generalisation that "some day it will be of use", which leaves one in some doubt since it might be swept away in the continual deluge!

Perhaps it is true to say that only the few can employ grains of sand, formulating them into bricks and actually build some edifice. Obviously there are all levels of research but a strong theoretical core must be developed that will bring all the parts together, since if this is not done, we shall be washed out to sea in an immense tide of unrelated information.

By all dictates of probability, the reader will have turned back the page, inspected the title of the thesis, and returned mystified on what possible grounds the growth of the skull has to do with this introduction. At this point I would like to indicate that the following pages should provide, at least, a number of grains of sand .... perhaps there may be a resemblance of a brick!

The word "Gestalt" conjures up a structural affinity, which is correct up to that point since Gestalt theory originated in the psychological field. The early use of this theory was in opposition to the now obsolete atomism of the associated theories. VON EHRENFELS postulated that the term Gestalten or configurations were physical states and processes, and that the characteristic properties and effects of these could not be achieved by putting together the

properties and effects of their so-called parts. In other words, a visual figure be it a skull or a chair is more than just an aggregate of little pieces. If one thinks of a chair, it consists of a number of sides, has characteristic shape, size and colour etc. If this chair is broken down to its constituent parts - and here I mean the cellular level or perhaps sawdust - then the force (for lack of a better word) which holds this sawdust together to form the visual form of a chair, is no longer. In short, the total sum of the parts, i.e. sawdust, does not add up to the original whole .... something is missing.

In the case of a cell PUTTER (1923) emphasised the Gestalt-character. For example, it is more than an aggregate of its parts because the individual parts are not capable of an independent existence. He went on to point out "that strictly speaking, it is the particular manner of the composition of the materials and processes, their spatial and temporal organisation which constitutes what we call life. What physiology teaches in addition to the physics and chemistry of living systems, is the theory of the Gestalt properties of these systems". Parallel development of the Gestalt approach occurred in this country with HALDANE insisting that "wholeness" was an essential feature of the organism. This theory, of course, has some snags because in a machine one can take all the pieces and reassemble whereas the structure of a living organism cannot be separated without loss of their properties.

Sufficient light has been thrown on the Gestalt theory in the above paragraphs to indicate the importance of the holistic approach to a problem. The fundamental character of a living organism is its organisation and disintegration of its single parts will give various aspects of knowledge but can never provide a complete answer or explanation to its "living". BERTALANFFY (1933) was fully aware of this point and stated "... the chief task of biology must be to discover the laws of biological systems to which the ingredient parts and

processes are subordinate. We regard this as the fundamental problem for modern biology".

Naturally, no single thesis can possibly encompass more than a small fraction of its field of operation at any time, but some effort must be made to give as wide a perspective as possible. The work is directed at the development of the craniofacial complex during a specific period of time and, as will be shown later, this particular period falls within a very intricate set of growth controls involving both the autonomous and pituitary, the internal (maternal) and external environments and other influences including the genetic endowment.

Thus the growth of the rat can be regarded as the Gestalt while the growth of the craniofacial complex is a part. In one sense, a great deal is known about growth and development of the rat so that one can only add a little to the knowledge already amassed - in short this thesis can be described scientifically as a study which "strengthens" the Gestalt, i.e. rat growth.

Having established the general description, the next problem is to endeavour to make some approach to the Gestalt. We have accepted that the growth of the rat can be regarded as the whole but while the skull has certain growth patterns specific to its development yet the growth of the animal, apart from these localised factors, is "centrally" controlled. On this premise it is possible to investigate and correlate the growth of the rat as a whole with the growth of the skull.

Examination of general body growth can only be accomplished in the present work by means of measurement, and two have been used to express the growth rate of the animal as a whole. Weight represents three-dimensional growth while length represents two-dimensional growth and both these dimensions have been used as a base line of general growth. Hence the growth of the craniofacial complex, i.e. the part, can be related to the body growth, i.e. the whole, so

that we have at least gone a short way towards satisfying some aspects of Gestalt theory. The value of this particular approach was amply demonstrated in work on the rice rat (Oryzomys palustris natator) (PARK, 1972) and patterns of growth were found which previously had remained undetected.

1.2 General Systems Theory and Growth

Essentially the term, Growth, denotes those changes of a living system that are manifest as measurable changes, and particularly as an increase in size. However, the increase of weight due to intake of water or deposition of fat can not be considered as growth. A more accurate view of growth would be to connect it with the essential living systems involved with metabolism since every living system must have continuous exchange - anabolism and catabolism of its components. From this point of view, growth can be defined as a quantitative increase of a living system which stems from the greater presence of anabolism of building materials over catabolism.

Within Systems Theory, the characteristic state of a living organism can be classified as an "open system". A "closed system" is one in which material neither moves in nor leaves. When a system is "open", there is an intake and output mechanism which brings about change in the components. Thus living systems are open systems (BERTALANFFY, 1969) which maintain themselves in exchange of materials from the environment, and in continual building up and breaking down of their components.

Having established that a living system can be interpreted as an open system, we must next examine the general characteristics which are applicable to open systems. According to the second law of thermodynamics, a closed system must attain a time-independent equilibrium state with maximum entropy and minimum free energy. In an open system, however, a time independent state may be attained being constant as a whole and in its phases, irrespective of the

continuous flow of the component materials. This particular state has been termed "Fliessgleichgewicht" by BERTALANFFY (1932) meaning a steady state.

Let us continue the development of this theory towards growth by remembering that in a closed system preservation of the equilibrium does not need energy but to perform work the system must never be in equilibrium but be trying to attain it. To ensure that this aim is achieved the system must be able to maintain a steady state. In short, the characteristic of an open system is the essential state which allows for the continuous working capacity of the organism.

The initial conceptualisation of the open system was related to the biological field by BERTALANFFY (1932) and he was to show a little later on that certain kinetic principles could also be linked with open systems. A parallel derivation of similar ideas stemmed from BURTON (1939) but only later the realisation emerged that the concept of open systems had been applied to thermodynamics by DeFAY (1929).

BERTALANFFY (1960) used a general transport equation to define open systems and from the solving of this equation three interesting points arise all of which were characteristic of steady states, but which were similar to those of organic metabolism. The first of these points showed that there was a maintenance of a constant ratio of the components in a continuous flow of materials; the second point showed that the composition was independent of, and maintained constant in, a varying influx of materials - this was ably demonstrated by the fact that in varying nutrition and a different absolute size, the basic composition of the organism remained constant. The third characteristic is that following a disturbance, the system eventually re-establishes its steady state - a type of adaptation reflex. From these characteristics it can be postulated that the property of "self-regulation" is an integral part of open systems.

Another characteristic stems from examination of conventional physical systems in which the state finally reached is defined by the initial conditions. Examples of this can be shown in closed chemical reactions where the concentrations appearing at the end depended originally on the initial concentration. When open systems are examined, the final steady state reached can be regarded as independent of the starting conditions, in fact the same endpoint can be attained from varying initial conditions or in different ways. This important characteristic is known as "equifinality" and expresses the profound difference between most inanimate and living systems.

The principle of equifinality has an important application in the growth of the whole organism especially within the experimental aspect of this thesis. In animal growth it is generally accepted that following a temporary cessation of growth and from varying initial sizes, that the ultimate species-characteristic size can still be attained. The one proviso is that damage to the growing tissue does not reach the extent of preventing normal function and growth when normal conditions return.

Most processes of growth have the property of equifinality and it must be remembered that growth rate can not be regarded as a function of time - it does not change direction and decrease as age increases - but is a function of body size. Thus, following some form of inhibition, i.e. undernutrition, the growth then resumes at the rate which corresponds to the body size and not the age reached. Experimentally one can induce litters of rats to remain partly inhibited in their growth by undernutrition (PARK, 1969) to the extent that there is a 50% loss of weight and 30% loss of total length relative to normally fed animals. Depending on the maternal capacity, these rats having been weaned and supplied ad lib with solid food rapidly recover. If the maternal capacity of the mother, however, has been unable to supply enough milk to prevent

damage of tissues, then recovery is only partial.

In a similar way, specific growth rate was found to be less in litters which were small and therefore having a higher weight, than animals in large litters and with a smaller birth weight (KOPEC, 1932) - these animals originating from natural litters.

Equifinality, however, is not only applicable to weight and size growth alone since it also exists the forming of organ balance as well as biochemical body constituents. Various organs and the size of the body must retain some kind of ratio if the whole organism is to work. We all know that widely differing stages of development exist in newly-born different species and that allometric growth will maintain a balance until the final phase is reached. An example can be seen in the brain and cranial development followed by the basal and facial components or, the gradual growth of an immature mandible, in the case of a rodent, to contain the dentition which has already reached full adult size.

Application of open system theory to the following study will be shown to be the basis of the fundamental life phenomena and will lead us to the feedback mechanisms which are responsible for the homeostasis. The complicated growth control of the preweaning and postweaning rats with its autonomous and pituitary divisions, the maternal and external environment, the maternal capacity governed by stimulatory factors, and the genetic endowment, is part of the feedback mechanism since it straddles across both the negative and positive sections thus making the growth spectrum cyclic.

1.3 Growth in Perspective

"Every living organism represents a hierarchy of organised systems" (BERTALANFFY, 1960) so that the study of animal growth as a whole, must by definition be envisaged from both different standpoints and levels. This

particular statement takes within its framework all types of growth such as tumour growth, increase of adipose tissue, epidermal cell replacement and many others. Thus the term "growth" is governed by the particular aspect of biological increase to which it is applied.

There are a number of important aspects relating to growth which must be briefly considered to orientate and give awareness to the nature and complexity of the subject. Admittedly a classification of any kind is the first and most primitive scientific approach, but the plethora of growth details entitle them to some attempt in classification before plunging into more narrower confines of detail.

1) Synthesis of species-specific substances: this means the production of high-molecular organic compounds such as proteins and, that their species, tissue, cell specificity are continued.
2) Factors for identical reproduction: the gene system is an example with widespread influences on cell processes and protein synthesis.
3) Cellular growth: an organism grows by means of cell multiplication, i.e. mitosis, together with cell-size increase and the production of intercellular substances.
4) Growth of whole organism: this type immediately implicates a large number of different structures but with dynamic processes which can be separated for recognition. In the early formative phases there are movements of cells closely resembling migrations and these are often described by a number of names such as invagination, gastrulation, etc. In logical sequence we than have the determination and separation of various embryonic areas under the phenomenon known as segration. Differentiation is another function of cells. Finally, we can introduce the term growth as the enlargement and multiplication of cells and subsequent increase in body size.

Thus it can be seen that there are many divisions in growth and with them are numerous definitions, the emergence of which is not at variance with the main theme but rather the intricacies of finer points and interpretations.

If we examine the following definitions it will be found that both are fully applicable to the study of the growth of the rat:

"Growth denotes those changes of a living system that are manifest as measurable changes, and in particular as an increase in size"

and

"Growth is an addition of material to that which is already organised into a living pattern"

Applying the term "growth" to the body of a rat we have, broadly speaking, a waxing and a waning which can be demonstrated by measurements. However, as was pointed out previously the weight of an animal could involve the normal growth, i.e. increase of muscle, bone, etc. and that of adipose tissue.

Because of these difficulties we can reclassify the whole animal growth process into three categories:

(a) True Growth

This type of growth is linked with time and if young rats are examined following birth, growth will be found to be "autonomous". This is followed a few days later by "autonomous + early pituitary" influence. Finally, as the weaning point approaches the growth control becomes wholly "pituitary". There are, by necessity, many other factors involved which can be broadly covered by the terms "maternal environment, external environment and the genetic endowment".

(b) Additive Growth

Essentially size can be increased by the intake of fluid, the

deposition of adipose tissue (beyond that required for average existence), the laying down of more shell, bone, cartilage, enamel, dentine and cementum. This type of growth differs from true growth because their processes vary in kind from the increase in the amount of living material itself. The presence of additive growth has, in the past, been the stumbling block to the formulation of a precise definition of true growth.

(c) Growth Regression

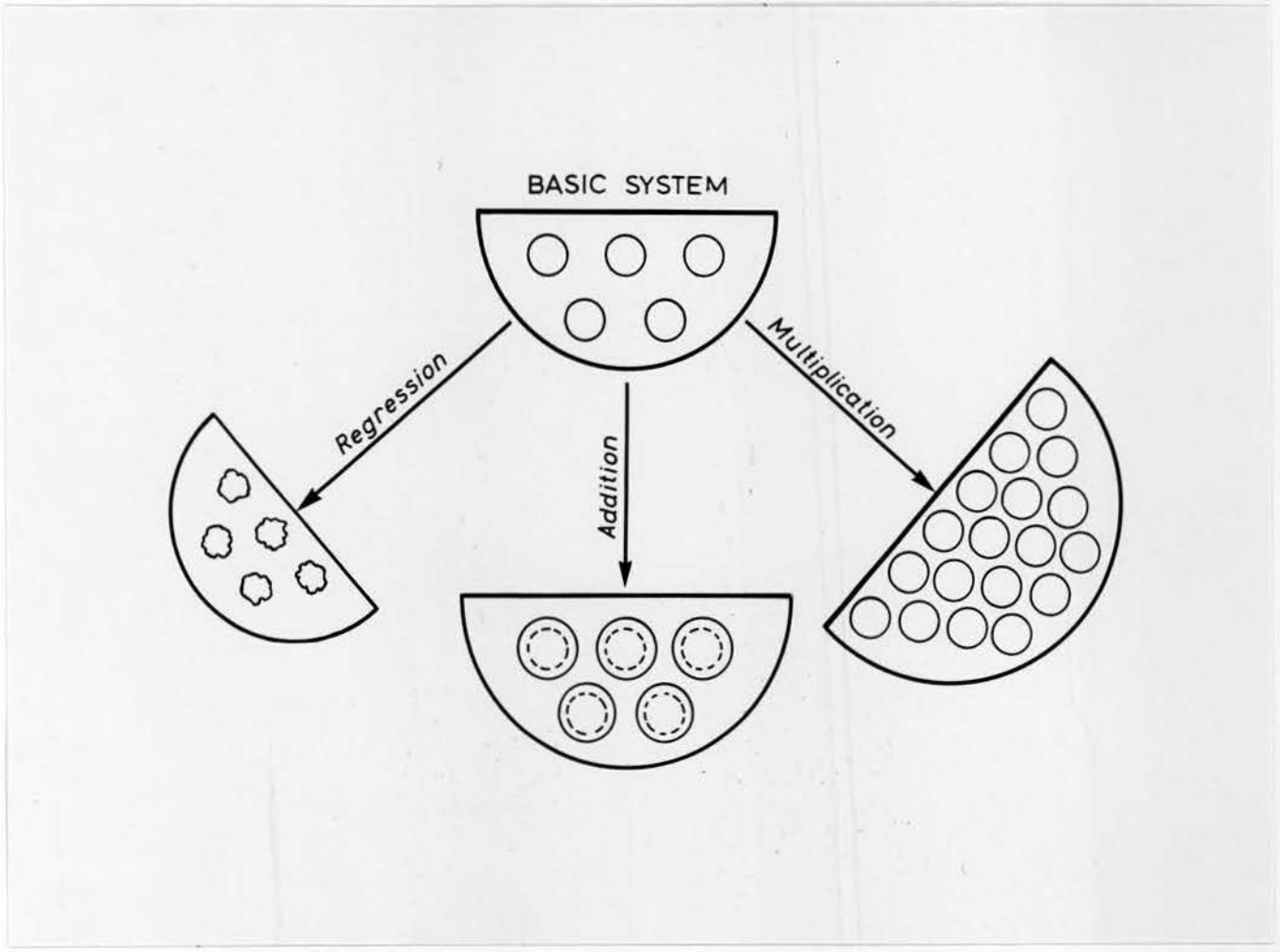
This is a looser term in which one embraces the changes in weight and size due to onset of senility or perhaps disease. With age, for example, there may be a decrease in weight and there are often height changes - many of which might be termed as postural defects but irrespective of the cause would have a direct bearing on any measurements.

The basic system leading to all these types of growth is shown in a diagrammatic representation in Figure I.

5) The Morphogenetic Process: development eventually leads to various shapes and forms and in the more embryonic phases the changes are mainly accomplished by formative movements. As the later stages appear the changes arise from relative growth which involves different growth rates in various directions and by various parts of the body.

6) Influencing Factors: the growth process has a large number of factors within its own boundaries which exert various influences, i.e. hormones, genetic factors, differentiation, ageing, etc. These could be covered by the term "Internal environment", which carries us to the "External environment" consisting of all those factors from without the animal such as nutrition, vitamins, temperature, population density, behavioural aspects, etc.

Figure 1 Schematic representation of the alternative modes of growth increase and decrease. A basic cell system can either increase by multiplication of its units, or increase the size of its units. Regression can imply reduction of the size of the units or a reduction of the number. In a particular organ where morphogenesis is complete and maturity reached, the influx of fat will increase the size of the organ but must be regarded as additive growth.



1.

1.4 Theoretical Growth Control

The regulation of organ and tissue growth has involved a great deal of speculation from which a number of hypotheses have emerged. It might be said that a large number of sophisticated tools of great precision have been used and in some ways they have outdistanced the concepts - in content they produce excellent results even though they may have been applied to a wide range of unconnected subjects the original problems of which have been formulated with much less precise phraseology.

The main aim of this study is directed at the morphogenesis of the skull during normal and altered environmental effects and thus some perspective must be attained of the recognised or contested theories of the growth control.

From a phylogentic standpoint, the allometric growth of any organ must be adapted to the needs of the organism and stemming from these genetic changes is the final form which has been urged along its path by natural selection. There are fluctuations in organ weights that indicate increases and decreases of function, and these fall within the physiological field.

One hypothesis which can be termed the "Self-inhibition" has arisen on the grounds that growth may be linked by negative feedback mechanisms with the production of specific tissue inhibitors. Basically the idea is that each organ produces growth inhibitors which are pushed into the extracellular body fluids (WEISS, 1955, WEISS and KAVANAU, 1957, KAVANAU, 1960). The postulate is that these inhibitors or antitemplates are capable of moving over cell membranes and can interact with complementary templates within the cell. It is thought that the templates stimulate cell growth but are unable to act if bonded to antitemplates. On this premise, a growing organ will produce an increasing number of antitemplate inhibitors until the situation is reached that all templates have been neutralised.

ROSE (1957) suggested that the attainment of an appropriate mass of any particular tissue, once reached, would inhibit its own growth, while BULLOUGH (1964, 1965) produced a similar postulate based on epidermal cells producing substances known as chalones which inhibited the mitotic process.

The "Self-inhibition" supporters do not regard cellular proliferation as being controlled by exogenous stimulators but that each organ produces its own inhibitor.

Another explanation was forwarded by TANNER (1963) linking overall body growth with both stimulators and inhibitors. This suggested that the inhibitors arising from the body were being "observed" by some controlling point outside of the organs being controlled. With the brain developing more rapidly than the body, the regulatory mechanism could be sited in the brain.

A variation of the TANNER theme was tabled by BURWELL (1963), BURCH and BURWELL (1965) and BURCH (1969). This particular hypothesis accepted that one particular tissue would act as a regulator and furthered this by suggesting that the lymphatic system represented the radar and produced inhibitors. The mass of each organ could be checked by the lymphatic system from tissue-specific substances (TCF), and if the organ should fall behind in its development, then the lymphatic system could produce stimulators called mitotic control proteins (MCP).

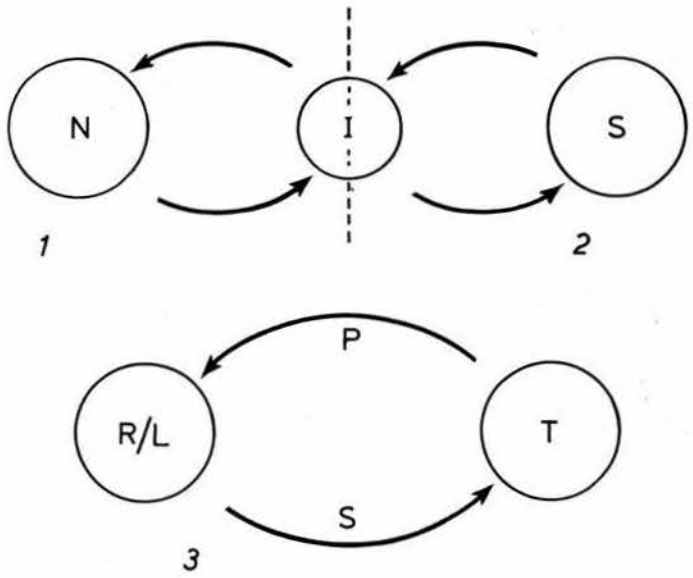
The theories so far described can be summarised in Figure 2, and it is perhaps better to leave the subject to those directly concerned with it.

The "Fundamental demand" theory has been with us for a considerable time and within the present century the idea that the activity of the organ was linked with its growth was postulated by MORGAN (1901). Later, the relation between atrophy and hypertrophy was brought forward by BOYCOTT (1929, 1932) in the sense of lack of use and use of the functional units. Further discussion emerged from the work of WALTER and ADDIS (1939) relating the work done and the eventual size achieved. One cogent phrase emerged from BRODY (1945) in which he stated that

Figure 2 Schematic representation of the theories of growth regulation. N, non-existent stimulation; I, inhibitor; S, stimulator; R/L, regulatory organ; T, target organ; P, product of target organ monitored by R/L.

1) An example of self-inhibition in the absence of specific stimulation (ROSE, 1957; BULLOUGH, 1964).
2) An example based on the antitemplates (I) inhibiting growth by inactivating the intracellular templates (S) in the organ from whence they originate (WEISS and KAVANAU, 1957).
3) Theory of exogenous stimulation, R being regarded as the brain (TANNER, 1963) and L represents the lymphatic organs (BURCH and BURWELL, 1965). Using the lymphatic organs (L), then P represents tissue coding factors while S represents mitotic control proteins.

THEORIES OF GROWTH REGULATION



"the organism changes geometrically so as to remain the same physiologically", and there is no question that there is a direct link between size and function. A number of reviews delving into the place of function and size has been undertaken over a fairly short period of time (WARBURTON, 1955; ABERCROMBIE, 1957; SWANN, 1958; PASCHKIS, 1958; GOSS, 1964; and BUCHER and MALT, 1971).

In the subject of control mechanisms, SWANN (1958) noted the feedback mechanisms and blood-borne factors and that the common denominator was their appropriateness to the function of a specific organ. This was followed by WRIGHT (1958) and a number of other workers such as GOSS (1964, 1965, 1966, 1967), all of whom supported the idea that tissues of the body increase or decrease depending on the usage.

As a proviso, there are some snags to this thinking. For example, how does one explain the growth of a body in terms of functional control before those parts become functional? Or, how some organs not in use still retain some vestige of tissue.

The problem remains complex, and it appears that a combination of the hypotheses may be the correct answer. Basically the tissues of the body can be divided into two main groups (GOSS, 1972). The first group "includes blood cells, most of the viscera, and all the endocrine glands". These, in fact support the body as a whole. The second group consists of "motor, sensory and integumentary tissues" and these are more autonomous in activity and have localised responsibilities. Thus we have two groups of tissues namely "Systemic" and "Local" and it is possible to find affinities with the two main groups of growth control, i.e. the stimulators-inhibitors and the functional demand.

Finally, growth control still remains unsolved although we have gone some way in understanding the processes. Many organs and tissues have a large part of their destiny controlled by function in the final determination of size.

Yet again, humoral factors are also prominent in growth regulation. This synopsis has dealt with growth regulators as related to whole body growth and provides some perspective in the subject prior to future discussion dealing with the craniofacial growth.

1.5 Nutrition and Growth

Changes in the growth pattern of animals during early postnatal life can be attributed to two main factors - the genetic endowment and the environment. For purposes of this study, the major variables implicit in the concept "environment" have been represented in practice by those of "nutrition".

The field of growth and nutrition is vast and within the limits of this work it is only proposed to introduce those facets which have a direct bearing on the observations and their discussions. A large portion of the field has been devoted to the effect of deprivation etc. of specific protein and fat metabolisms, the role of enzymes, carbohydrates and the reader is referred to a recent review of the subject by WINICK (1972).

Generally, many of the nutritional deficiencies which would affect the foetal animal also have similar affects on the pre- and postweaning animal. However, if this state of affairs remained as simple as written, then the problems of normal development could be solved very easily. The intricacies of nutrition and growth can be brought into perspective by remembering that the science of developmental nutrition recognises three important interrelated parameters of under nutrition. These can be simply stated as: "Severity, duration and timing". The question of the age at which undernutrition occurs has brought into the developmental aspect the idea of transient periods of raised sensitivity. The term "vunerable" has been coined by DOBBING (1968) when relating the effects of undernutrition of body growth and brain growth with the lasting deficit and structural distortion of the latter. This particular term of "vunerable" is

probably more acceptable than the word "critical" or "sensitive".

The maintenance of rapidly growing uterine young makes demands on the female which alters her nutritional requirements. The developing rat needs energy to maintain its own metabolism and has further needs to enable it to increase its body mass. The mother, on the other hand, also has a number of demands above normal to contend with such as extra carriage of weight etc. As would be expected, during pregnancy there is a rise of food intake by the mother. However, if the food level is low then the embryos will often be found partially resorbed. This is not the place to discuss mechanisms of embryo resorption, but it is interesting to note that the triggering stimulus may be very similar to that observed in the bank vole (Clethrionomys glareolus) which showed nearly total embryo resorption linked with a population explosion.

Buffering mechanisms against the effects of undernutrition can be employed by the female rat. A typical instance is found in deficits of calcium in the food. During both pregnancy and the lactational period the female rat corrects the deficit of calcium by draining it from her bones. Most of the other minerals are present in some quantity. There are two other substances, namely carotine and Vitamin E, both of which have effects on the size of litter and death of the embryos when deficient (SADLEIR, 1969).

Maternal malnutrition can have deleterious effects on both embryonic and preweaning growth - basically this can be called the "maternal environment" but can be subdivided into two. In the preweaning division of the maternal environment RUSSELL (1948) has shown that immediately following birth to 22 days there was a greater intake of food by the mother and this was shown by the 279 calories needed to maintain lactation compared to the 79 calories of the embryonic period.

A simple fall in the food supply, either as milk or solid, sets off a

highly complicated chain of responses. The time factor is also important since various organs have differing growth rates and differing times of starting. For example, organs like the heart develop early whereas the craniofacial complex has a considerable way to go. The point when undernutrition begins to have an effect on the actual growth process depends, therefore, on the state of development reached at that time. WIDDOWSON (1968) reviewed some of the facts relating to growth and undernutrition and noted that under normal conditions there was an increase in the skeletal muscle with age and a decrease in brain as a percentage of the body weight. While normal growth shows changes in the relative weight of the organs, the biochemical constituents also change and at different rates. Many of these can be nearer their adult composition at birth than others and, as will be discussed later, specific constituents such as DNA in the brain of a rat reaches its full complement around 16 days following birth.

The academic importance of the subject lies in its relation to the manner in which bodily, organ and tissue growth is organised, and how the "program" of growth can be manipulated and distorted - often to a point of permanence - by restrictive influences in certain early stages. However, clearly this is not a purely academic problem since if too much tissue damage is sustained then the recovery is not possible and deficits may be left of both physical make-up and functional activities. This has been illustrated by DOBBING (1968) in rats where if the body growth is retarded at the time of the brain growth-spurt, there is a resulting growth deficit which resists subsequent nutritional rehabilitation. The presence of good brain growth obviously has an interdependence on the initial body growth.

In the case of the craniofacial bones there are different rates of growth and the three main divisions of facial, cranial and basal grow as individual

units as well as consisting of individual bones each having their own particular growth potential. Bones are always less highly mineralised at birth than they are in adult life and therefore there is a gradual increase with age. Undernutrition retards the maturation process of the bones (DICKERSON and WIDDOWSON, 1960). Bone is formed of an organic matrix consisting largely of protein and collagen, and it is on these that the calcium is deposited. The ratio of calcium to collagen in the bone makes a good index of the degree of calcification of the protein framework. Although undernutrition retards the maturation rate, the more marked effects are those of body length and particularly the weight. Weight remains the most sensitive and hence most variable of the three.

1.6 The Environmental Influence

The role of the environment as related to the regulation of organisms is a complex subject, but one often dismissed by the limited perspective of many of those whose research takes them deeper into their subject. The use of material tends to form its own horizons and when animals supply this material it is suffice for many to accept that these animals are of the same inbred strain and that a "standardised" laboratory upbringing has been adhered to. Naturally, the material obtained under such conditions may well be ideally suited to the requirements insofar as the conditions do not alter the constituents or nature of the material. However, when the subject is one directly aimed at the growth process, the environmental conditions take on a new importance.

JUSTUS LIEBIG (1840) laid the foundation for increased understanding of the role of the environment in the regulation of organisms. From his extensive work in the field of organic chemistry and its applications to agriculture and physiology emerged the now well known "Law of the minimum" which states:

"Of all essential materials needed for growth and reproduction of an organism, that available in amounts most closely approaching the critical minimum needed will tend to be the limiting one determining the organism's distribution and abundance". This particular law has been expanded to include factors other than nutrients, but these are better included under the "Law of tolerance". Basically, the "Law of tolerance" extended LIEBEG's law to bring in the limiting effects of the maximum as well. The "Law of tolerance" was originated by BLACKMAN (1905) and can be defined as "Organisms have an ecological minimum and maximum, with a range between, which represents the limits of tolerance. Absence or failure of an organism can be controlled by any factor approaching the limits of tolerance, i.e. a limiting factor". Further, it is to the credit of BLACKMAN that although he did not develop the concept, he did make another postulate which later became known as "factor interaction".

Although these "laws" were first applied to physical and chemical environment, and in the case of LIEBEG, primarily with plants, it has been extended to include animals (DAVIS, 1966).

Animals reared within the confines of the laboratory world are brought up under environmental influences peculiar to that world, but with the removal of many of the factors normally experienced outside, new factors emerge which eventually form a laboratory environment with its attendant problems. Some of these factors, i.e. those such as stress, take time, inbreeding to form a stable strain, control of cage population density, food, etc. These environmental factors are the more dramatic type but there are many factors present which, although not dominant, nevertheless have considerable effects on reproduction, maternal behaviour, maternal capacity, weaning and growth.

Once within the confines of laboratory settings, the animals can be subjected to a number of controlled environmental changes. These changes can

induce variable growth reactions but such is the complexity of growth that the changes observed do not necessarily give insight into the pathway by which they have been attained. Thus one can reduce the weight of an animal by reducing the food intake - this is observable and measurable - but the actual body chemistry can only be postulated until work on the specific mechanisms, if this is possible, has been accomplished.

A number of methods exist by which growth pattern can be altered and these all fall into the category of environmental whether naturally existing, such as nutritional problems, or experimentally induced, such as exposure to radiation. The following classification gives some indication of the possibilities to be considered:

1) Variation of the existing (normal) plane of nutrition.
2) Diseases.
3) Change in the physical environment such as temperature extremes, humidity, oxygen levels, etc.
4) Administration of hormones, various drugs, irradiation, etc.

1) Variation of the plane of nutrition.

Two problems arise immediately, firstly the young in embryonic and preweaning states rely on the maternal support system, thus variation of the plane of nutrition cannot be direct since it involves the mother - preventing her from feeding or reducing her own intake; secondly, the variation of nutrition of all animals following weaning is direct, that is the amount of food can be controlled more expertly than in the first group. The two groups, however, not only differ in their nutritional control but also in their stages of growth, and in the rat the preweaning period of growth covers some very important craniofacial developments.

Animals beyond weaning have been studied by careful control of the food

over selected periods of time (HATAI, 1907; OSBORNE and MENDEL, 1914; JACKSON, 1925; McCAY et al, 1939; McMEEKAN, 1940; McCANCE, 1960; PETERSEN and BAUMGARDT, 1971). This particular older group of animals is of interest in the present work but from the point of view of "recovery" from previous nutritional deficits.

The embryonic growth does not fall within the present scheme although the effects of differing normal birth weights of the rats forms the base line from which the control and experimental observations are made. A certain amount of work on malnutrition has been done by altering the food level of the mother. The majority of investigators imposed food restrictions during the pregnancy without altering its quality (CHOW, 1964; CHOW and LEE, 1964; BERG, 1965; LEE and CHOW, 1965; HSUEH et al, 1974). Other workers gave pregnant rats an unlimited food supply but containing only a little protein (SEEGERS, 1937; NELSON and EVANS, 1953; CURTISS, 1953; RICHARDSON et al, 1964; VENKATACHALAM and RAMANATHAN, 1964; ZEMAN, 1967; WIDDOWSON and COWEN, 1972; MENAKER and NAVIA, 1973). These types of nutritional deficit, especially when of a severe level, led to a reduction of the number of young in the litter, and to a small size at birth. The young born under these early conditions also grew slowly irrespective of good supplies of milk.

Control of nutrition during the preweaning period of development can be approached from two angles. The first method is to vary the size of the litter being fed by the mother, while the second method involves the removal of the mother from the litter, thus reducing the amount of feeding time (JACKSON and STEWART, 1920; EAYRS and HORN, 1955). The amount of technical assistance required to employ the second method would certainly curtail "popular" use, but more important is the possible loss of heat and other stress factors involving both young and mother.

The first method, variation of litter size, forms an important part of this thesis and will therefore be discussed at length elsewhere. However, the reduction of litter size is a common practice among those searching for an optimum state. The method has been extended by means of the random cross fostering procedure of KENNEDY (1957a, b) and WIDDOWSON and McCANCE (1960). This was achieved by having a number of litters born within the same few hours, the litters were mixed together to distribute as evenly as possible any potential genetic differences in growth before selection and formulation into small and large litters and assigned to a foster mother. The result was that the mother of a large litter had her lactational capacity stretched resulting in undernourished young.

The principal disadvantage of the large litter concept is that there is no control over the individual nutrition so that changes could be due to the lack of food linked with the physical inability to overcome competition of littermates. If we accept that food in a large litter has an uneven distribution we can expect to find differences in the growth pattern. For example, the largest rats of the males and females could be regarded as the most competative and dominant. Those in the middle ranges of weight and length represent the average competitors, while those most retarded could be regarded as the least competitive. Unfortunately, the problem does not rest merely on competition or dominance since there are other important variables such as the maternal capacity - the proven mother - and the mammary function. Behind the mammary function lies further complications such as the "milk-ejection reflex" (CROSS and HARRIS, 1952) which depends on stimulatory factors.

For experimental purposes it must be accepted that the large experimental litter will produce a number of growth changes, and that for purposes of description they could be placed into categories of high, medium and low, thus

representing the very small, the medium sized and the large. The division of the groups can often be difficult but even allowing for that the total litter, even comparing its largest litter members, still exhibits marked changes in its growth pattern compared to the control litter.

On odd occasions, it is possible to find a "proven" mother having a high lactational capacity and thus some of the heavier members of her litter could be very similar to some of those observed in a natural litter. This problem arises because of the number of births per female are not related to her innate capacity to feed. Thus we have the possibility of having a natural litter which is too large for that particular mother.

The question then emerges: what is the most acceptable size of litter which will allow the average female to maintain milk supplies and allow the individual members of the litter to reach their maximum growth potential? If this question can be solved - even partially - it will go a long way towards setting up a "standard" litter for use as a control. Two experiments have been formulated as part of this thesis to attempt to answer the problem since the literature covers control litters of sizes ranging from 2 - 9. The lower range probably falls into the "overfed" group while the upper range could fall into the "deficient" group, depending on the mother's lactational capacity. Within natural selection the external environment plays a major part in the "survival of the fittest" and in the sheltered laboratory world the seemingly large natural litters would, in all probability, be lessened in numbers in wild conditions, thus leaving the mother less to feed.

A more detailed discussion concerning problems of maternal environment in relation to litter size will be found elsewhere.

2) Diseases.

This is a wide complex subject which, although having a considerable

retarding effect on the growth pattern, does not involve the present work. It might be pointed out, however, that acute starvation and infection can either singly or together form a basis of growth retardation.

3) Change in physical environment.

A full review of the various physical alterations is not required in the present context but it is important to remember that BERGMANN's and ALLEN's "Rules" have influenced the interpretation of animal biological adaptation (ALLEE and SCHMIDT, 1951).

The effects of temperature changes on subsequent growth of body weight and length and skeletal development has been undertaken by a number of workers who showed, among other things, that a relatively simple change could induce changes (SUMNER, 1915; OGLE, 1934; SCOW, 1944; ASHOUB, 1958; HARRISON, 1958, 1963; HEROUX and GRIDGEMAN, 1958; JOHANSEN, 1962; CHEVILLARD, PORTET and CADOT, 1963; ROUBICEK, 1966; BARNET, 1965; RAND, BURTON and ING, 1965; STEEGMANN and PLATNER, 1968; LEE, CHU and CHAN, 1969).

The relationship between growth and the environment is closely linked with situations producing stress from over-crowding, noise and aggression, etc. and it was noted that there appeared to be a positive correlation between body weight and skull development which was observed to be maintained, but at different levels, in two groups of animals living under very different conditions (DIAMOND, ROSENZWEIG and KRECH, 1965).

4) Administration of hormones, various drugs, irradiation, etc.

Most methods involving hormones and drugs for the modification of growth are specific in action, and are mainly directed at the analysis of various mechanisms. Growth changes by means of administering ACTH have been studied (MOSS, 1955; JOHANNESSEN, 1965) but with the influence on the endocrine systems there are alterations in food intake, etc. so that not only are the conditions

varied but only specific effects are also recorded. This particular approach requires a wide knowledge of endocrinology and biochemical body synthesis and the specific nature of the influences.

1.7 Variables in Cranio-Facial Growth

Growth problems can only be solved if account is taken of the multiplicity and changes of the interrelations. In fact the animal should be regarded as a dynamic structural totality. There are, however, a number of factors which exert influence on the transformatory processes of the cranio-facial bones and their interrelations during both the embryonic and postnatal phases. Generally these can be divided into two main groups based on the synopsis of SCHUMACHER (1972):

1) Local Factors
   - (a) Dura mater, cerebrum, organa sensuum vasa, nervi.
   - (b) Musculi: masseterici, faciei, linguae, colli, pharyngei, nuchae, epicranii.
   - (c) Pneumatisation, dentition, respiration.
   - (d) Phonetics, mimics.
   - (e) Mechanical factors, behavioural habits, loss of teeth.

2) General Factors

   Genetic endowment, constitution, nutrition, sex differences, vitamins, hormones, climatics, domestication, psyche, statics (posture, body size, gravity).

As with many factor synopses one can use other terminology or express a complex number of factors by using only one covering word, thus we can rearrange the factors into 13 groups:

1) Nutrition.
2) Mechanical factors.
3) Constitution, domestication.
4) Psychosomatic factors.
5) Work.
6) Statics - posture, body size, gravity.
7) Pneumatisation - bone marrow.
8) Brain.
9) Musclature.
10) Speech
11) Dentition.
12) Foetalisation.
13) Genetic

The meanings behind both these groups are identical and they represent the forces present which influence the various cranio-facial changes through time.

Lists of variables can continue to extend as more knowledge emerges so that eventually one is faced with a mass of data which appears to have no firm outline. The main factors controlling the cranio-facial morphogenesis following birth can be divided into (1) Genetic; (2) Local environmental influences.

LIMBORGH (1970a, b, 1972) defined the genetic role by pointing out that the beginning must arise from the "genome" which has the various genes necessary for the morphogenesis of the cranio-facial structure, i.e. the hereditary characteristics. Although genes are regarded as inherent to the cells, the range of their influence can differ considerably. Some genes, for example, have a limited action which is confined within the cell structure. If the genes determine the cell potentials, then the tissue which is composed of cells are part of the effect. Influences of this type can be called "Intrinsic genetic factors". There are, however, genes which extend their influence beyond the limits of the cells or tissues to which they belong. These particular genes do not express themselves within their own cells but reveal themselves in the reactions of the tissues they are exerting influence upon. In short, these are genetically determined factors having a kind of remote control of distant action. These are regarded as "Epigenetic factors".

These two types of genetic control can be subdivided in their influential roles as 1) Intrinsic genetic factors with a local effect.

2) Epigenetic factors with a local and general effect.

The epigenetic factors have a local control when they arise in adjacent structures while when produced by distant structures have a general influence. As examples the effect of growth hormone on the epiphyseal cartilage can be termed a "general" effect while the degree of muscle development can be regarded as "local" effects.

Outside the two factors produced by the genome lies the third controlling factor - the "Environmental". This can be divided into general and local depending on the degree of response to the influence. Within the present field of study the term "environment" can be used in a number of ways. For example the "Maternal environment" implies the nursing and lactational capacity of the female, whereas the term "External environment" implies all those factors of temperature and solid food which the young rat is influenced by. Naturally, unless the mother is removed at a defined time, these two environments would naturally overlap. The removal of the mother at 20-21 days does not solve the problem since biological weaning is a gradual process of slow fall of milk supply and gradual increase of solid food. The complications of the "environments" in relation to growth will be discussed in the various sections.

Cranio-facial growth controls appear to stem more from the effects of local factors than general ones and in LIMBORGH's words "They condition or modify the morphogenesis as governed by the genome".

The present work accepts the results of the genetic endowment and the environment on the postnatal skull morphogenesis in the rat under standard laboratory conditions, thus forming the control. To enable comparisons to be made, certain measurable points are selected to transpose a static picture of

growth at one moment in time of a dynamic process. The measurements are compared to a similar group obtained from the experimental animals at the same moment in time. The experimental animals will have undergone morphogenic changes induced by the alteration of the maternal capacity during the preweaning period. These environmental restrictions are lifted in the postweaning stage by unlimited food supplies so that the effects wrought by undernutrition on the skull morphology can be examined, and the recovery potentials assessed.

1.8 Concepts of Cranio-facial Morphogenesis

At the present time the concepts of cranio-facial morphogenesis stem from three "schools" of thought originating in The Netherlands, France and Germany.

The Dutch concept has its origins and stimulation from the work of VAN DER KLAAUW (1945, 1950, 1948-52) which, having been introduced in 1945, was expanded into a monumental article published from 1948 to 1952. During this four year period the Dutch Archives of Zoology (Arch. Neerl. Zool.) were entirely devoted to the documentation of the theory that the form of the skull is determined mainly by the functions of the brain and functions of the face.

The principle of holism lies behind this concept which considers the parts in an organism the members of a totality or whole - the Gestalt. This approach to biological problems has been supported by various workers (CUVIER, 1805; SIMPSON, 1945; BERTALANFFY, 1960). VAN DER KLAAUW saw the significance in relation to morphology and the application of it fell to DULLEMEIJER (1956), MOSS (1960) and MOSS and YOUNG (1960).

VAN DER KLAAUW introduced two ideas, the first that analysis should start with a proper distinction of the parts in a whole, and that this distinction should be based on the elements forming a functional unity. He divided the skull into 84 separate functional cranial components, and described how the function of each component, together with its spatial relationship with other

components, largely determines its size and shape. Using VAN DER KLAAUW's meaning, all functional elements that participate in a function can be termed "functional component" but in his thesis only the skeletal parts were incorporated. Later, the soft tissue was brought into the concept by DULLEMEIJER (1956, 1958) and MOSS (1960) and this has been followed since.

The second idea, mentioned above, is the acceptance that the functional components are dependent on eath other in various ways.

MOSS (1971) summarised the work of VAN DER KLAAUW by stating: "The essentially operational concept of functional craniology began with the recognition that the several functions of the head and neck are carried out by what have been termed functional cranial components. These components, in turn, are formed by (1) all those "soft tissues" (muscles, glands, spaces), i.e. viscera and visceral spaces related to a specific function, and (2) those related skeletal tissues which serve to protect and/or support these "soft tissues".

This particular terminology was altered slightly by new formulations of MOSS and YOUNG (1960), MOSS (1962) and MOSS and GREEBERG (1967), and a given function is now regarded as being carried out by a functional matrix which is supported and protected by a skeletal unit. The two can then be amalgamated to form a functional cranial component.

The importance emerging from this modified terminology is that the main skeletal unit is, in fact, secondary to the functional matrix in the sense of size, shape and spatial position. A great deal of previous work dealing with biometrics does not appear to correspond to the growth and function concepts. This is because of the skeletal unit being secondary to function. It follows that once the genetic endowment has been expressed in the original osteogenic centres, the growth and morphogenesis subsequently following must be environ-

mentally determined.

Two major changes in outlook have stemmed from these new concepts and no doubt others will follow in turn. MOSS (1971) pointed out that the sutures could no longer be accepted as primary growth sites, and this has been supported by a number of investigators (MOSS, 1954, 1964; SELMAN and SARNAT, 1957; WATANABE et al, 1957; GIRGIS and PRITCHARD, 1958; SARNAT, 1963). In fact, sutural growth appears in a very new light, being a follower of the primary growth of the soft tissues, i.e. the neural and facial tissues. Thus sutural growth is secondary and because it depends on its stimulation to come from soft tissue increase, it is therefore compensatory. To this description can be added the words of MOSS (1971) that the sutural growth was a "mechanically obligatory event" since it relied on soft tissue expansion.

The new concepts have re-stimulated thought regarding the importance of the condylar cartilage which had hitherto been accepted as being a growth centre of prime position which was responsible for most of the growth. The new ideals suggested that this condyle could be a single unit relatively independent of the rest of the mandible. Experimentation has supported this postulate and the mandibular condyle can now be regarded as secondary, compensatory and mechanically obligatory since it shows a natural reaction to the passive lowering of the mandible due to general skull development.

Work continuing the VAN DER KLAAUW concept has been in most instances successful and it has opened new avenues. DULLEMEIJER (1956) and SCOTT (1955) rose to the stimulation and gave impetus and this was followed by further work (DULLEMEIJER, 1958a, b; DRYER, 1961; GARN et al 1963; HOFER, 1965, etc.).

The French school and its concept first emerged from the efforts of DeLATTRE and later FENART (DeLATTRE, 1951; DeLATTRE and FENART, 1956a, b, 1958, 1960, 1963-64). The concept is potentially very applicable to the

clinician since it is based on the idea that the plane of the lateral semicircular canal is the only correct physiological point about which all aspects of phylogenetic and ontogenetic skull growth should radiate.

What the French school have achieved is to replace the already existing arbitrary registration lines such as the Frankfurt Horizontal Plane, etc. with a point which is valid both physiologically and biologically. The work has continued in various forms with considerable interest from both the clinical and experimental worlds (MOSS 1958a, b, 1959, 1961; FENART, 1966).

The German school has been in similar fields in its efforts to find methods of registration but, unlike the more solid front of the French school, their ideas are still in the gelling form but with gathering momentum and the signs of a shape and course. A review of the field has been presented by HOFER (1965), and MOSS (1971), summarised the concept as follows: "The most conservative (phylogenetically and ontogenetically) portion of the neural mass is the brain stem. Accordingly, the most conservative portion of the cranial base will be the cerebral surface of the postsella portion of the skull base (clivus), which supports the immediately overlying brain stem". Most skulls have a characteristic bending of the cranial base often known as basilar kyphosis, and the question therefore arises on whether the registration point should be placed on the pre- or postsella part of the cranial base. Measurements based on the postsella registration are employed for taxonomic purposes while presellar registration has been applied to human material. This is dependent on the anterior cranial base remaining unchanged following the completion of the olfactory lobe and tract of man by the late prenatal or early postnatal stages. The original use of this method stemmed from De COSTER (1951, 1952) and has been employed by many others (KRAUS et al, 1959; MULLER, 1960; HOPKIN, 1963; PROROK, 1963; ADUSS and PRUZANSKY, 1964; BJORK, 1966; MOSS, 1955, 1956, 1958, 1959).

1.9 Regulation of Cranio-facial Growth

In the structuring of the background of the proposed work we have been introduced to some of the major variables concerned with cranio-facial morphogenesis and acquainted ourselves of the main current concepts.

Some mention must now be made of the regulatory mechanisms - or postulated ones - which are associated with skeletal growth.

Basically, the skeleton consists of bone, cartilage and fibrous connective tissues interlocking in various combinations to form the whole - in the case of the skull its primary function is to protect and support the other tissues.

In terms of growth control, the true amount due to the genetic endowment remains relatively unmeasurable since we are faced with the influences of the environmental factors. As MOSS (1972) suggested "There is a general consensus of opinion that both cartilage and bone arise from a common stem cell whose intrinsic genetic information is responsive to extrinsic factors in the microenvironment for the determination of its ultimate pathway of differentiation". It is interesting to note that the concept of direct genetic regulation of bone formation and its growth had been supported by bone grown using culture and implantation methods (GRUNEBERG, 1963; SAWIN and HAMLET, 1970). However, further consideration by FELL (1969), who pointed out that development in isolation was only applicable to the early cartilage and that in true bone the mechanical and environmental variables carried an important role, has since brought more balance to the concept.

The inductive interactions in bone morphogenesis are closely related to the genetic endowment (reviewed by HOLTZER, 1968; OWEN, 1970) and the progenitor cells are thought to have prior genetic instruction which effectively allows them to respond to unductive influences outside the cell. In the craniofacial development there is evidence of inductive interactions between certain

areas of brain and the mesenchyme which ultimately forms the intramembraneous and endochondral calvarial bones (HALL, 1971). However, evidence is sparse in supporting the intrinsic-genetic factors as directors of bone growth - especially in its final form. This supports the contentions of FELL (1969).

Regulating influences cover the remodeling of the bone and cartilage which occurs as the general growth continues. Two suggestions have emerged from the work of HARRIS and HEANY (1970). For descriptive purposes, which in reality returns us to "systems theory" they postulated control loops with negative feedback characteristics influencing the reshaping of the bones. The first postulate involved hormonal control in which they indicated the parathyroid hormone and calcitonin, while the second brought in the environmental mechanic forces. Naturally, the hormonal effect is systemic while the mechanical forces are local. For greater detail and discussion on the subject of homeostatic remodeling and the suggestion that the mechanical factors modify in some way the response of the bone to the systematic stimuli, the reader is referred to McLEAN and URIST (1968) and HARRIS and HEANY (1970).

A new concept has arisen which may explain how a biomechanical negative feedback loop could function and thus explain how a biomechanical stimulation is met by a change of bone structure. BASSETT (1972) pointed out that some of the plepexities of the skull development could be answered by knowing that electrical phenomena and correlating them with bone morphology. By means of orthodontic forces applied to teeth he found the electrical polarisation and linked it with cell behaviour. For example, experiments were made on the long bones and histological evidence has shown that osteoblastic activity, concavity net compression and electro-positivity are all connected with one another. On the other hand, the converse is true since osteoclastic activity, convexity net tension and relative positivity are closely linked. Experiments showed that artificially

induced electrical currents produced bone accretion at the negative pole while resorption occurred at the positive pole in long bones and mandibles. This was first introduced by BASSETT (1964) along with co-workers PAWLUK and BECKER. The finding was confirmed by others and was shifted from the osteoblastic to the osteoclastic activities (MINKIN et al, 1968; O'CONNOR et al, 1969; YARRINGTON and JACQUES, 1969; PAWLUK and BASSETT, 1970; WITTELBOL, 1970; FRIEDENBERG et al, 1970). At a macroscopic level, ignoring the mechanisms involved, WOLFF's law still holds and can be currently stated: "The form of a bone being given, the bone elements place or displace themselves in the direction of the functional forces and increase their mass to reflect the amount of functional forces" (BASSETT, 1968). Orientation of the bone structure to the force has been examined by many workers of which perhaps D'ARCY THOMPSON (1943) will be remembered along with more recent investigators such as FROST (1964), JOHNSON (1966), BASSETT (1966, 1971a, b), ENLOW (1968). The BASSETT hypothesis links mechanical and hydraulic forces, electrical polarisation and specific cell specialisation in a cybernetically based, negative, feedback control system.

Originally, the cranial growth was regarded as being a combination of three factors: the production of morphogenetically primary expansive forces within sutural tissues and which pushed the bone apart; that similar forces existed within the cephalic cartilages; and that there were processes of deposition and resorption of bone. This has now been proved as not feasible and the "functional cranial component" (MOSS and YOUNG, 1960; MOSS, 1962; MOSS and GREEBERG, 1967) has emerged. The functional cranial component consists of two entities, the first of these is the functional matrix which carries out the function, while the second is the skeletal unit which protects and sometimes supports the functional matrix. Two types of functional matrices have been described, namely periosteal and capsular. The periosteal can be demonstrated by muscle, whereas the capsular type consists of the cephalic functioning spaces such as the oral cavity,

nasal and pharyngeal spaces, the neural and orbital masses, etc. The periosteal matrices regulate the size and shape of the bones to which they are related. The rise and fall of functional demands are the primary stimulators and these are followed by the secondary, compensatory and mechanically obligatory bone changes. This is supported by the formation of the angular process of the mandible which depends on the activity of the medial pterygoid and masseter muscles. There is a progressive loss of shape etc. if nerves are damaged or muscles ablated going right down to resorption. If, however, the muscles show hypertrophy the form of the angular process increases. This is transformative growth.

The cranium can be taken as a good example of capsular matrix action. There is an expansion of the brain mass during early development which causes the neurocranial capsule to expand. The cranial bones are not pushed apart by sutural forces or cartilages, but are passively floated apart as the capsule within expands. This can be regarded as translative growth.

The bringing in of further postulates, at this point, will not serve the original intention which was directed at the major principles and current ideas on the subject of growth and the skull. Further comments will be reserved for discussional purposes elsewhere.

1.10 Aims of Investigation

It is always tempting when introducing an area of investigation to cull appropriate quotes from antiquity in order to show that the particular problem under review has concerned eminent scholars throughout the ages - and thus establish its importance, as well as give the reader confidence in the scholarliness of the author. Apart from adornment, which many quotes owe their selection to, there are others which not only fit the subject, but give hints

as to the greater perspectives involved - to write about which would add yet another volume to the saga.

When the subject matter deals with the importance of growth of the cranio-facial complex, then a plethora of references is available. What is striking, however, is not that the importance of the environmental factors during the pre- and postweaning period has been commented on and possibly discussed for centuries, but that in spite of these many references to the systematic experimental laboratory investigation of the problem is no more than .... old.

If a few of the contemporary journals of research are read then the reader is being influenced (unknowingly) by an editorial decision which occurred not so many years ago. The sheer productivity of the increased numbers of research workers forced the editors to either eliminate or drastically curtail one of the essential parts of any research report - the review or historical introduction. Nowadays one is forced to come directly to the point and the setting of it receives the terse but immortal phrase "In a previous communication .....". What have we lost? We have lost a valuable source of historical perspective and in the world of the journal there is little chance of it returning. Only in the thesis is some attempt still made to give a place of the ensuing problem in its historical context. Thus the little grain of sand which has been uncovered might be given an opportunity to fit into its correct spatial relationships in terms of others.

The history of cranio-facial research shows a cumulative advance (mainly stemming in the developmental side from the biological approach) by building up a body of research findings, theories, procedures and techniques which are passed on. Because of the passing on of the knowledge, i.e. between generations, one can therefore say that there is a tradition of cranio-facial research. Within this general tradition may lie several specific traditions, tending to blend but

showing enough characteristics to be separately identified.

The introduction of this thesis remains by circumstances limited in its historical sense, but the sections within it are purposely directed at making those readers who are involved in specific fields fully aware of the complexities of cranio-facial growth and some of the scientific and philosophical extensions of them.

The aims of this thesis can be given in a few sentences but there is no doubt that they cannot be fully realised - the further into the subject we delve, the greater the horizon becomes. We can but reach towards it!

Aims:

1) To study the relation of the maternal environment on the growth of the cranio-facial skeletal complex during the preweaning phase of development.
2) To assess the specific growth rates of the bones of the facial, cranial and basal groups in relation to each other and under verying maternal influences.
3) To study the relation of the maternal environment on the growth potential and gradients of individual bones with a view of establishing the degree of sensitivity.
4) To correlate the "principle of equifinality" by examining the postweaning phase of development and establishing the degree of recovery and rate of recovery from the states induced during the preweaning phase.
5) To ascertain the effects of the maternal environment, following inhibition, on the subsequent formation and eruption of the dentition.

"The progeny of research is research and many exciting problems have yet to emerge. There is little doubt and every hope that the closer we come to experimental solutions to many of these problems, the closer we come to the understanding of the behaviour of organisms, regardless of the species".

LEVINE 1962.

PART II

MATERIALS AND METHODS

"The main conclusion is that measurements are rarely sufficiently precise to distinguish between the possible alternative relations which could be fitted to them and that in any event, the gross curve probably represents the summation of many contributory processes and only by chance approximates to some simple relation also with a single biological meaning".

NEEDHAM 1964

2.1 Experimental Animals

The consideration of the type of animal to be used stemmed from a number of reasons. For example, the use of primates would have given little extra information but would have introduced problems of cost, space and time. Of the larger animals such as pigs, dogs and cats the problem of time and numbers again forms a barrier. Essentially, growth experiments need multiparous animals if the control of nutrition is to be achieved by litter size manipulation. The rodent has been the most acceptable of the laboratory approach for a variety of factors:

1) At birth the developmental state reached is early and in many cases only comparable to states found in utero of many other animals.
2) The size of the litters is large, and in addition, the rat and mouse both make excellent foster mothers and new litters or extra members are readily accepted.
3) Both breeding and growth is rapid making longitudinal studies possible.
4) The skull of the rodent is typical of the mammalian type being of the thin-walled variety analogous to the human.
5) Within 30 days following birth the development of the calvaria is almost complete as also is the dentition.
6) The variables of size of animal, standardisation, management and expense are all well within the resources of the small animal unit.
7) In both rat and mouse, the preweaning period ceases biologically by 20 days - although most young rodents will feed on milk if available so that the maternal capacity by litter manipulation can be altered and the recovery observed in a period of 40 days. This period could be followed further but has been selected to coincide with skull development and the completion of a functional dentition.

The decision was made to use albino laboratory rats (Sprague-Dawley strain) because of the factors stated above and the wealth of literature available on the subject. A further supporting factor was the knowledge gained from previous work (PARK, 1964, 1969, 1972).

The rats used in this study were obtained from a random breeding stock population maintained in the animal unit of the Dundee Dental School. Two breeding nuclei were formed - an expediency stemming from problems of time, accommodation, and the time available for processing - and produced 618 control and 1,000 experimental young. A further 340 young were produced, but due to various discrepancies, were not used.

2.2 Colony Management

The animal unit of the Dundee Dental School is of modern architecture (1966) and has been designed for optimal conditions of animal care. Ventilation is controlled by an air-conditioning system which maintains the general environment at 23-25°C with a relative humidity of approximately 50%. Every room receives natural light from one end but during winter they are further illuminated by fluorescent lighting during the hours of 09.00-17.00.

The rats were kept in ordinary wire rat cages (44 x 28 x 12 cms) with a food basket and water bottle at one end. Bedding consisted of fine gravel and mothers were supplied with thick cleansing paper during pregnancy which they expertly shredded and formed nests.

Food consisted of a standard type known as Rat Cake Diet 86 (North Eastern Farmers Ltd., Bannermill, Aberdeen) and was composed of the following ingredients:

Diet 86	Content %
Ground Wheat	50
Ground Barley	26
White Fish Meal	7
Meat and Bone Meal	6
Dried Yeast	5
Salt	1
Dried Grass Meal	5

In addition a Vitamin Supplement was supplied:

8 million I.Us. Vitamin A

2 million I.Us. Vitamin D3 per ton

2.3 Experimental Design

Prior to the main investigation two experimental animal groups were established aimed at finding the size and range of the litter giving the best standard growth. The theory behind variation of litter will be considered elsewhere. The main experimental animal group was formed of control litters (based on the findings of the two experiments above) and large experimental litters.

1) Experiment I. The Influence of Numbers.

Based on the alteration of litter-size at birth by the random cross-fostering method (KENNEDY, 1957a, b; WIDDOWSON and McCANCE, 1960). Litters consisting of young rats numbering from 2 to 15 per litter were established. In litters of 2, three replicates were formed, while in litters consisting of 3, 4, 5 and 10, two replicates were formed.

Following the formation of the litters at birth, the first 24 hours was regarded as Day 1 of the series - total coverage being birth to 20 days.

2) Experiment II. Maternal Capacity and Stimulation.

As in Experiment I, this is based on the random cross-fostering method and a number of litters formed. Throughout the period of observation, which lasted from birth to 20 days, a sequence of changes in the number of rats contained in each litter were made at three definite stages. Each experimental litter was designated by a code letter ranging "A" to "R". The three stages were initiated at 5, 10 and 15 days after birth:

On Day 5: Litters coded A to E were reduced in numbers as follows:- A to 4, B to 6, C to 8, D to 10, E to 12.

On Day 10: Litters coded F to K were reduced in numbers as follows:- F to 4, G to 6, H to 8, I to 10, J to 12. A further litter coded as K was also reduced to 12 to provide a replicate.

On Day 15: Litters coded L to R were reduced in numbers as follows:- L and M to 4, N to 6, O to 8, P to 10, Q and R to 12.

3) Main Experiment.

The material was obtained from a 24-hour series of rats formed on the random cross-fostering principle into control and experimental litters. The control litters were based on the results of the two experiments above, i.e. the litter size which allowed the best possible utilisation of the young rat's growth potential - the so-called "standardised litter". The series covered a time interval of birth to 40 days. A total of 618 control and 1,000 experimental rats were used.

2.4 Breeding

Adult rats were formed into breeding units of 36 females and 12 males with each cage being occupied by 3 females and 1 male. The distribution of males with females was completed within the same hour and the "family" thus set up was

left until pregnancies were well advanced whereupon the male was removed and the mothers changed into breeding cages with nesting material. The aim was to ensure the greatest numbers of litters being born within the same few hours. Observation of the families made sure of birth times as well as checking on the maternal ability. Records of each rat were kept concerning matings, weight during pregnancy, number of young born, survival rate, maternal ability and general condition.

Once a number of litters were born within the same 6 hours, they were carefully removed from the mothers and mixed together. The mixing was undertaken to distribute as evenly as possible any potential genetic differences in growth before random selection and formulation into their experimental litters and in many instances foster mother. After mixing, the young rats were sexed, and formed into groups of 6 (3 males and 3 females) and returned to each mother as control (small) litters. The large litters, designed to show the effects of undernutrition on growth, were formed in a similar manner into litters ranging from 13 to 17 with as equal a distribution of the sexes as possible.

2.5 Selection

The material investigated was obtained from a 24-hour series of albino laboratory rats (Sprague-Dawley strain) covering a period extending from birth to 40 days. This selection was based on allowing the first 20 days (preweaning period) with its particular changes due to undernutrition, to have the same length of time for recovery although any recovery might well require further time. For purposes of calculating the age of the animals, the birth of a litter was accepted arbitrarily as 1.00 a.m., unless there were indications of a very recent birth upon which adjustments were accordingly made. Other births were noted as they occurred.

2.6 Division of the Sexes

The basic principles used in previous research regarding the separation of the sexes during the first 20 days following birth (PARK, 1969, 1970, 1972, 1974) were applied in the present study. Studies on the differences of male and female growth rates during the first 20 days (preweaning) have produced no significant results (SWANSON and VAN DER WERFF TEN BOSCH, 1963; PARK, 1970; PARK and NOWOSIELSKI-SLEPOWRON, 1971). Sexual differences beyond 20 days, however, showed a transitional phase up to 25 days followed by manifestations of weight and then length differences, so that by 30 days the sexual division was significant to the eye. Because of the doubts as to the point in time best to select as the watershed, the more popular weaning time of 20 days was selected. Thus the initial few days following day 20 do not show any particular differences of weight and length even though biochemical differences do exist.

Other tangible differences of growth rate between the sexes during the preweaning phase have been examined and, in particular, the eruption of molar teeth. KONIG and MARTHALER (1958, 1960) made investigations and could find no conclusive support of a sexual difference in eruption time. These findings were obviously not significant, but it must be remembered that any differences of eruption time must be related to the background of eruption variation - which, as we all know - can be considerable.

Within the framework of the various findings one can only accept that any sexual differences which exist within the preweaning phase must lie with the range of growth variation and for purposes of this work can be regarded as not of significance.

2.7 Specimen Preparation

On the selected days within the series the litters were removed from the cages and the rats killed by administration of an overdose of ether. Various

linear measurements were made of the body within some 20 minutes of death and the weight was recorded. The heads of the animals were removed and immediately placed into a solution of 2% calcium acetate/10% formalin at a pH of approximately 7. Many of the older skulls had the skin removed to facilitate the entry of the fixative.

Maceration of the skulls was first attempted by means of 1% KOH followed by 30-45 minutes in the ultrasonic cleaner bath (SPENCE and TONKINSON, 1969), then degreased in acetone and finally bleached in 5% of 100 vols H_2O_2. This method was extremely useful with the older skulls but contained a drawback when applied to younger skulls. In the case of young rats, the ultrasonic waves tended to separate the individual bones from the skull along the suture lines. As a direct result of this "disintegration", a longer, but non-disrupting method was used for the rats by immersing the heads in 1% KOH for approximately 3 days followed by the removal of the flesh, de-cerebrating and degreasing in acetone.

In the soft growing regions such as the osteodental fissure, condyle heads etc., the removal of soft tissue by instruments could inflict considerable damage to the tissue. The tendinous insertions at various points also proved troublesome since if force was applied a tendency existed whereby the bone around the insertion or point of origin tended to lift out.

The problem of shrinkage was noted in the very young skulls as well as the bony edges of the osteodental fissures. As some measure of prevention, the skulls were never allowed to dry out on the bench until the various parameters had been recorded. Once cleaned, they were replaced in specimen bottles with fixative fluid. Older skulls did not show any signs of shrinkage but for purposes of continuity of data, were also immersed in fixative. Any errors caused by swelling were small compared with the distortions found in the young dried skulls.

Examination of the individual bones forming the cranio-facial complex and the drawing of their morphological features necessitated the disarticulation of the skull. The ultrasonic waves were tried upon a number of skulls and it was found that after 50 minutes some of the bones had fallen away from the skull whereas the majority of the remaining bones could be disengaged by fine tweezers. All skulls subjected to this disarticulation were initially measured as part of the major investigation and following disarticulation, the individual bones were laid on filter paper for examination.

Specimen preparation was found to result in a number of skulls being damaged, although some premature disintegration was due to open sutures. In such instances extra litters were always bred as an answer to this contingency and the whole litter replaced in the project. Although the replacement system was carried out a small fraction of the total number of skull measurements were made from damaged or missing bones, such damage or distortion during any particular measurement has been noted in the records.

2.8 Radiographic Methods

The principles involved in the present work originally stemmed from the use of a Hilger X-ray unit during 1961-63. This particular unit had only been applied in the biological field in the University of Edinburgh to a few pilot studies since its main use was directed at metal analysis. The Hilger X-ray unit consisted of a continuously evacuated X-ray tube and a 40 μgun of EHRENBERG and SPEAR design (1951) with a focal area of 0.04 mm diameter which was reduced by a viewing angle to 0.04 x 0.004 mm; a filament of tungsten with a current of 4.1 A, a copper target oil-cooled, the spot line-focussed and the X-ray take-off angle of 6 degrees.

Although the results obtained by use of this unit were satisfactory for a flat piece of bone, the problem of obtaining an accurate image of developing

teeth within a mandible or maxilla could not be approached from the unit design. The attainment of satisfactory X-rays of human teeth has been based on CIESZYNSKI's "Rule of isometry which is based on a geometric theorem stating that two triangles are equal when they have two equal angles and a common side." To disregard this rule results in either elongation or foreshortening of the X-ray image. To produce accurate images the X-ray beam must be projected to strike teeth at right angles and in the clinical X-ray unit the cone of the unit can be manipulated to the correct angulation. In the Hilger X-ray unit the micron gun was not capable of angulation or any movement.

The correction of the beam alignment to the molar teeth of a rodent mandible was partially solved by the construction of a cassette which could be angulated to the beam (PARK, 1964, 1966, 1967).

During further work on the mineralising pattern of rodent molars (PARK 1970, 1972, 1974) it was found that the natural tilt of the molar teeth in the mandible - the greatest tilt of the occlusal surface to the median saggital plane - returned the most meaningful image of the occlusal surface with the least superimposition. In the maxilla there is a corresponding tilt of the occlusal surfaces laterally and outward.

In the mandible the pattern of mineralisation was, therefore, more clearly represented without change of the angulation. Naturally, this approach is feasible for the study of the general pattern of mineralisation but not if measurements of root length were involved since foreshortening and elongation would be present.

The Hilger X-ray unit was operated at 30 kV, 3 mA for 30-60 minutes using maximum resolution plates (Kodak).

The expertise gained from using the Hilger X-ray unit has now been transferred to a Philips P.W. 1008 self-rectifying table model generator with a capacity

of 27.5-50 kV adjustable in 2.5 kV steps with 4-40 mA continuously variable. The tube was of the fine focus type of 800 watts maximum, with a copper anode and nickel filter. Two collimators were designed to fit the unit and their use depended on the size of the specimens - the smaller collimator had dimensions of 45 x 1.8 cms while the larger was 54 x 5 cms.

Two types of film were employed in the present work. Kodak Kodaline standard film (Orthochromatic film 2698 on a 0.1 mm Estar base) was used as experimental scanner since its exposure times were within 5 minutes. For closer study Kodak experimental scientific film V6028 with its fine grain allowed the production of finely detailed negatives and photographs but with a longer exposure time of approximately 20 minutes.

2.9 Bone Mineral Analyser

Effective scanning of the mineral content of skulls of rats which had undergone nutritional deficiencies together with controls was accomplished by means of the Norland-Cameron Bone Mineral Analyser. This particular unit is in the experimental stage and has been linked with the Medical Physics Unit of the Dundee Royal Infirmary where it was on loan from Cambridge for a short time.

The immediate use of the Norland-Cameron Analyser was directed at patients where disease has some effect on the mineral content of the bone. Its design was aimed at measuring automatically and displaying two specific values - bone mineral (in grams/centimetre) and bone width (in centimetres).

In the clinic the equipment required three procedures before scanning:

1) Calibration.
2) Positioning of the patient.
3) Scanning.

At the end of the scan the instrument delivered direct digital readouts of

bone mineral content and bone width.

The equipment consisted of a Scanner Module and a Computer Module.

Scanner Module

The traversing scanner assembly passes over the limb or specimen with its gamma ray detector. Moving with the detector, the sealed ^{125}Am source (usually 200 millicuries), located below the stainless steel deck, provides a collimated beam of monoenergetic photons which passes through the specimen. In very thick bone the higher energy of ^{241}Am is needed but the ^{125}Am source is known to give more accurate results (Fig. 3).

Computer Module

This is an electronic system incorporating the circuitry necessary to automatically control the scanner, establish the baseline values, read the attenuation of photons, and calculate the values of bone mineral content and bone width.

The Scanner Module had three operating speeds:

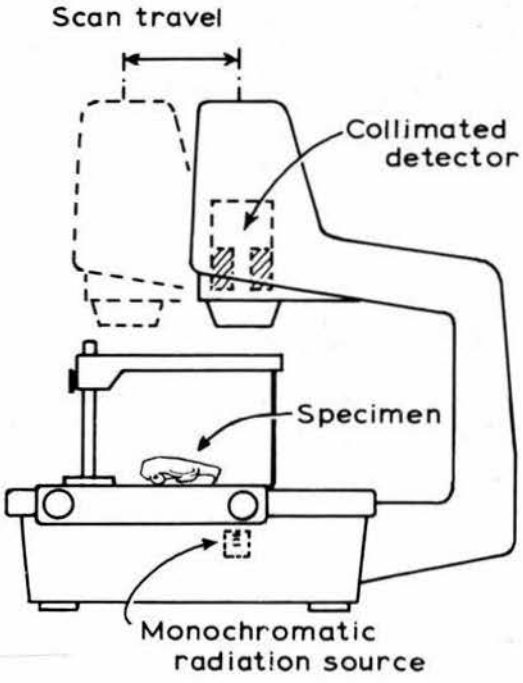
1) Rapid search at 1 centimetre/second.
2) Scan at 1 millimetre/second
3) Scan at 2 millimetres/second

In order to ascertain the mineral content of the control and experimental skulls a pilot experiment was set up with varying speeds of scan and in a longitudinal and transverse plane. The computer module gave a figure recording which, as expected, was too limited to use for comparisons.

A decision was reached that two modifications would be required prior to scanning further skulls. Firstly a recorder was linked to the computer module to allow the scan to be recorded as a profile - suitable for both measuring and photographing, while secondly, the scanner was recalibrated for the lower mineral content of the skulls compared with human arm.

After calculation, the profile recording of each skull was found to be twice the dimension of the specimen. The main peaks of the recording are accurate

The NORLAND-CAMERON BONE MINERAL ANALYSER



SCANNER MODULE

relative returns of the mineral content while the small peaks can only be regarded as within the range of error.

Both the longitudinal and transverse scans were arranged to pass over the same region of the skull. The width of the operational scan of the skulls was 5.0 mm. Within the preweaning period of development 204 skulls were scanned which included equal numbers of both control and experimental.

2.10 Nomenclature of Dentition

Terminology used in morphological descriptions of rodent molar teeth depends on the trend of the interest. On the evolutionary aspect the terminology is based on the Tritubercular Theory. This system stems from known homologies although there are times when they have been used to indicate the position of a cusp without resorting to the actual homology. The other interest lies purely in identification of the various morphological features of the teeth and is aimed at simplicity.

Simplicity is not the keynote of the evolutionary nomenclature which is difficult to handle and covers much more detail than is necessary in eruption studies. Examination of the recent extensive work of HERSHKOVITZ (1962, 1967) on the dynamics of rodent molar evolution only serves to stress the plethora of detail that exists. The possible confusion can be underlined when it is noted that in both upper and lower molars there are 40 separate designations of cusps and 15 folds.

For simple morphological description the most recent method of HUNT, ROSEN and HOPPERT (1970) appears to give the most satisfactory solution. The general major features of the teeth are regarded as tubercles (T) and, as they occur in pairs, the natural sequence is to recognise them as "T1, T2, T3 ....". In 1972 I pointed out that the nomenclature was perfectly adequate to use, but that a question of semantics arise since a "tubercle" indicates a swelling whereas a

"cusp" was specific for sharp points on the brinding surfaces of teeth.* Thus, it was suggested (PARK, 1972) that the so-called cusps should be renamed as "C1, C2, C3 ....". Other molar features such as transverse fissures are indicated by "Tf" followed by a number such as those found in the first mandibular molar - Tf1 and Tf2.

The terminology of HUNT et al (1970) include 5 sulci on the occlusal surfaces of the first and second mandibular molars and which are identified by "A, B, C, D and E". Finally, the supplementary cusp such as that lying buccal to the disto-buccal cusp or T6 of the first mandibular molar, was denoted as "v", i.e. a vestigial tubercle, while the disto-additional cusp or median tubercle of the first molar was marked by the letter "H" thus standing for "heel".

The nomenclature to be used in the present investigation are presented in Figures 4, 5, 6, 7 and cover all the molars both maxillary and mandibular. The basic system is that devised by HUNT et al (1970) with some minor alterations, as suggested earlier (PARK, 1972). Changes to the terminology include the replacement of the letter "T" by the letter "C" to represent the cusps; the supplementary cusps designated as "H" and "V" by HUNT et al are given the letter "T" with a number; the original practice of giving letters to the sulci has been retained and extended to the maxillary and mandibular third molars.

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* CUSP (L. cuspis, point) - one of the protuberances on the grinding surface of a tooth.

CUSPID (L. cuspis) - a spike.

CUSPIS (L. a point) - cusp

TUBERCLE (L. tuberculum, dim. of tuber) - a swelling.

STEADMAN's Medical Dictionary, 20 edit. 1961

Published: E. & S. LIVINGSTONE Ltd., Edinburgh.

Figure 4 Ventral and dorsal aspects of the first, second and third maxillary and mandibular molars of the albino laboratory rat. The first and second molars represent a rat aged 23 days, while the third molars represent a rat aged 30 days. Cusps represented by the letter "C" and appropriate number.

Rattus norvegicus

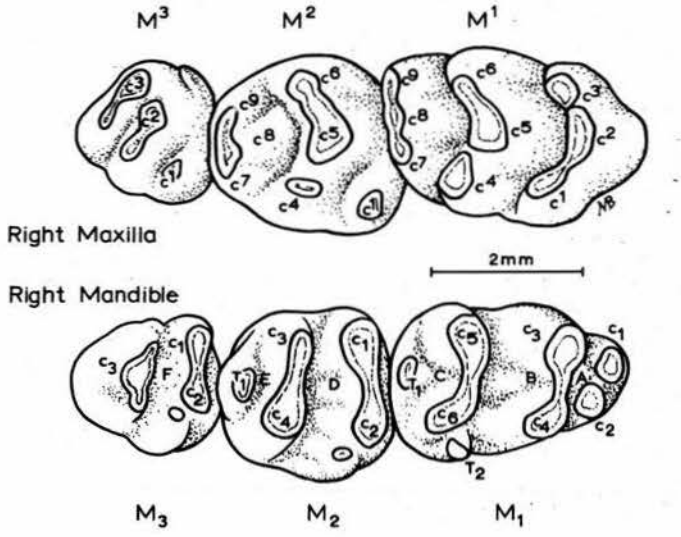


Figure 5 Lateral view of the maxillary and mandibular molars showing fully developed roots at the age of two months. Roots represented by the letter "R" and appropriate number.

Rattus norvegicus

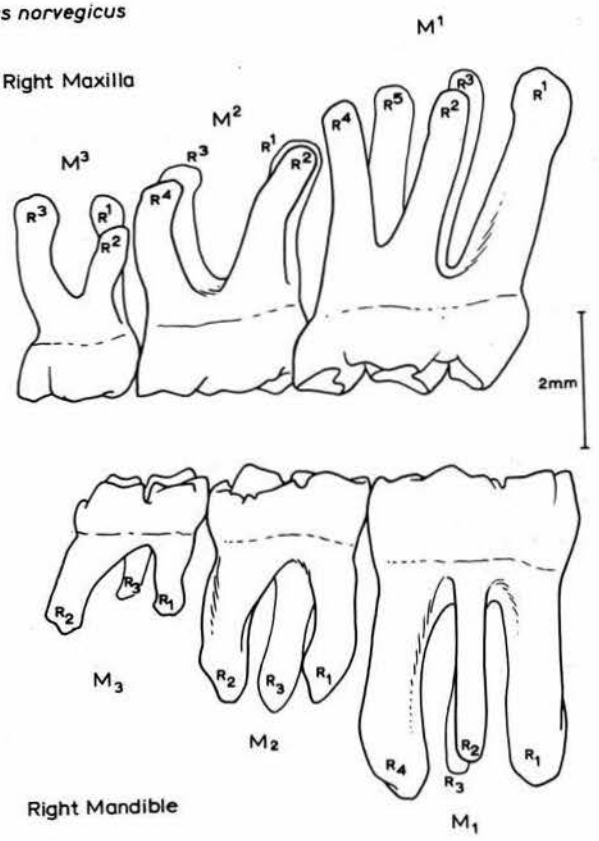


Figure 6 Cross sectional view of the molar roots taken through a plane sited at the upper third root level and showing relative diameters and positions. Roots represented by the letter "R" and appropriate number.

Rattus norvegicus

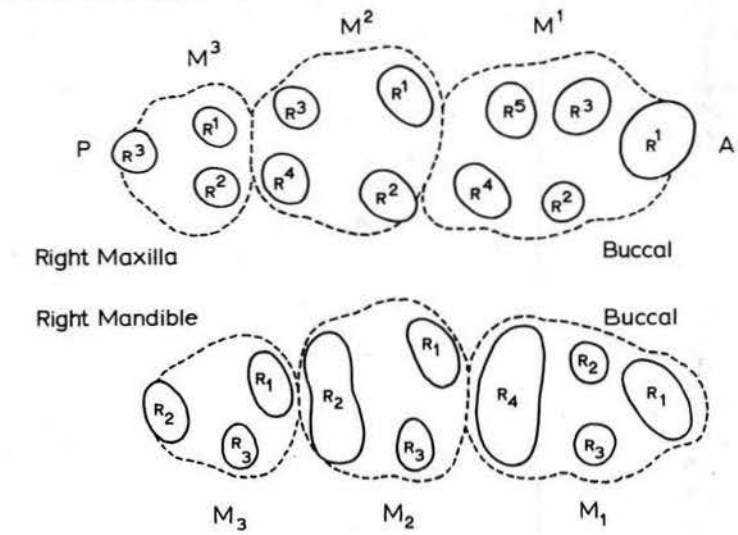
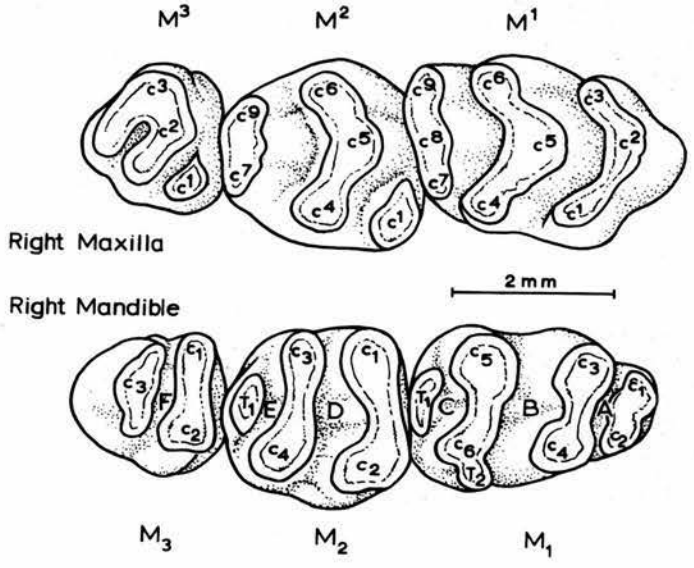


Figure 7 Ventral and dorsal aspects of the first, second and third maxillary and mandibular molars of the albino laboratory rat, 1 year old. Note the loss of characteristic cuspal morphology.

Rattus norvegicus



To obtain an immediate recognition of cusps originating from lower teeth to those originating from upper teeth, the number of the particular cusp mentioned is placed above the letter, thus "C^1" is the first cusp of a maxillary molar, while "C_1" represents the first cusp of a lower molar. The evolutionary absence of the first pair of cusps found in the first maxillary and mandibular molars - is not recorded for the second and third molars since the investigation is only related to the morphological characteristics, and not with their possible derivations.

For descriptive purposes, the root system of the maxillary and mandibular molar have designated with the letter "R" and a number. As in the cuspal system the number has been placed in a position depending on whether the tooth is a maxillary ("R^1") or mandibular ("R_1").

The generally accepted tradition of referring to all maxillary molar teeth by the letter "M" and a number, i.e. "M^1, M^2 and M^3; and that of referring to mandibular molar teeth as M_1, M_2 and M_3, has been retained.

2.11 Cranio-facial Nomenclature

In morphological description a name should be attachable to a specific whole or part and in the study of skulls all the parts have been awarded individual recognition. There are, however, different systems of nomenclature and in the case of the rodent, the latinized veterinary terminology of ELLENBERGER and BAUM (1926), which was designed specifically for four-footed animals could be applied. Basically correct but doubtful, if the dearth of Classical education in our training is presumed, that the flow of description would come easily to hand.

The alternative, however, is that used in general medical and dental teaching and hence communication - the Basle Anatomical Nomenclature commonly known as the"B.N.A" (JAMIESON, 1916). In the descriptions that follow the nomencla-

ture is that derived from the Birmingham Revision (B.R.) of the Basle Anatomical Nomenclature (B.N.A.).

2.12 Body Parameters

The relation of body weight and size (the whole) to the maternal environment and other factors are important indicators of influences which may extend to the cranio-facial complex (the part) and individual bones. Weight can be regarded as representative of 3-dimensional growth while length represents 2-dimensional growth. From successive weighings of individual rats or from linear measurements of rats of known age and sex a series of connected points as a function of age can be plotted. From this data also, it is possible to gauge the effects at various times of the inhibition of the maternal capacity and, later, the rate of recovery.

Naturally there are limitations in what is measurable and there are limitations in accuracy, but allowing that the degree of difference between control and experimental animals be sufficiently marked, then fine measurements will not add greatly to the significance of the results.

The following body records were obtained:

1) Total body length (snout to tip of tail).
2) Head-body length.
3) Length of tail.
4) Weight.

These records were obtained immediately following the killing of the litters by overdosing with ether. Each rat was weighed and the results taken to one decimal place in grams. Calculations of length were found by means of a modified Western Reserve measuring board (ACHESON, MACINTYRE and OLDHAM, 1959). Two such boards were constructed suitable for the young preweanlings and the post-weanlings and proved effective in previous work (PARK, 1969; 1972). In both

boards the tail holes passed through the end-plate which was of a thickness of 3 mm. The tail length was the only measurement involved with the end-plate and, since it was constant, 3 mm was added to each result to give true tail length. It should be pointed out that the tail length of a rodent should never be used for any purpose in itself but only to aid other requirements such as calculating the head-body length. The reason for its unsuitability as a measurable entity is due to the considerable variation in the number of caudal vertebrae in the rodent tail. A less important factor - only because it is visible to the observer - is the shortening of the tail due to stress conditions arising in the cages.

In the sections dealing with length, all the measurements have been recorded irrespective of their degree of usefulness. Further to this, although division of sexes was deemed not necessary within the preweaning phase, the sexes have been correlated with the measurements throughout the series.

2.13 Skull Parameters

To enable the morphological changes of the skull to be recorded for comparative purposes, a total of 22 measurements, based on the points illustrated in Figure 8, were selected so that the three major bone groups involved in the cranio-facial development were represented. These major groups consisted of the facial group, the cranial group, and the basal group which included the mandibles. Measurements were made with special external comparators (Fig. 9) and draughtsman's dividers. The dividers were controlled by a small cog wheel which allowed manipulation by one hand while the other held the specimen. All measurements were undertaken with the support of a dissecting microscope and the results checked for repeatability.

The following datum parameters were selected:

Figure 8 Sketch representing the datum parameters selected for studying the craniofacial complex.

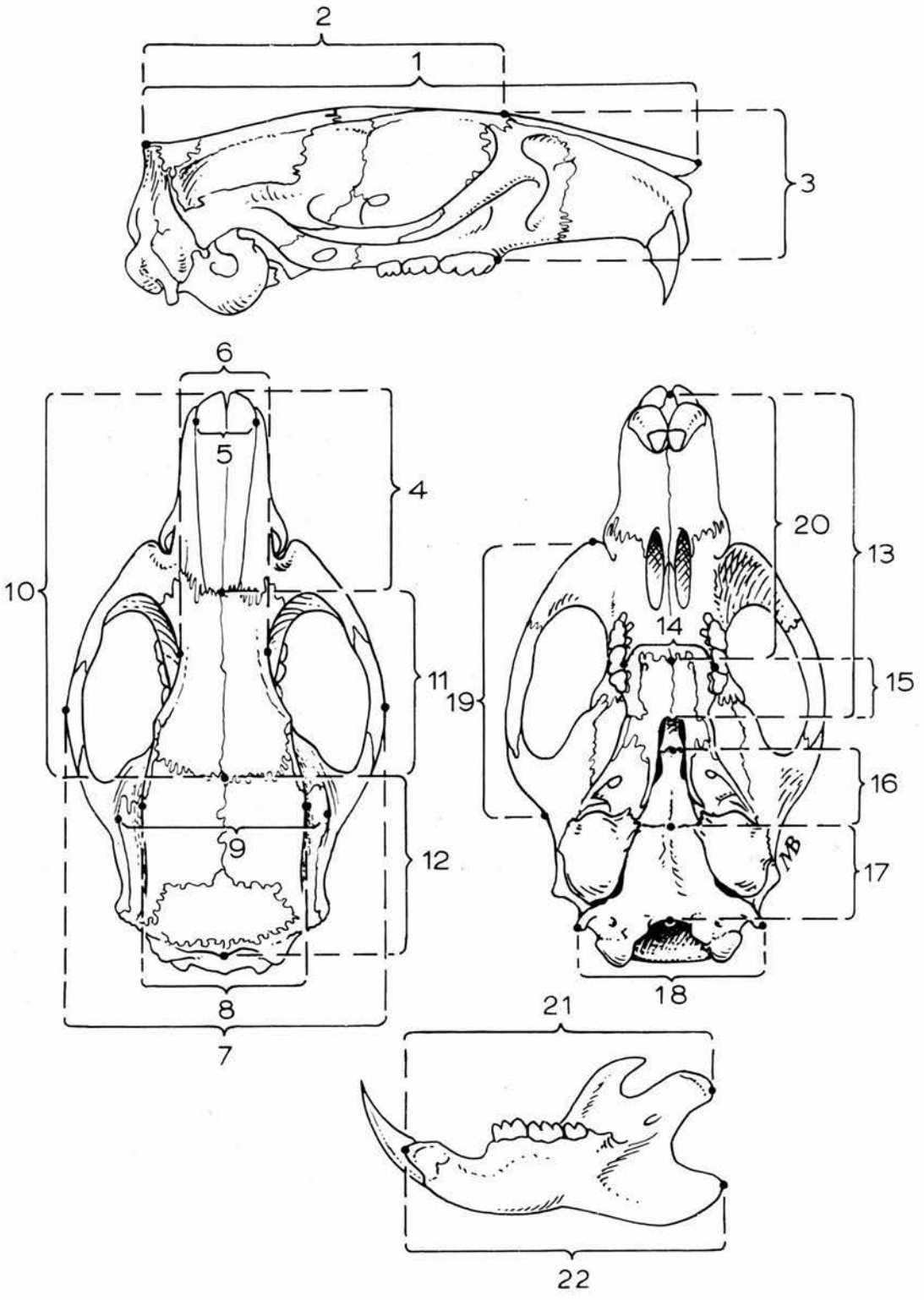

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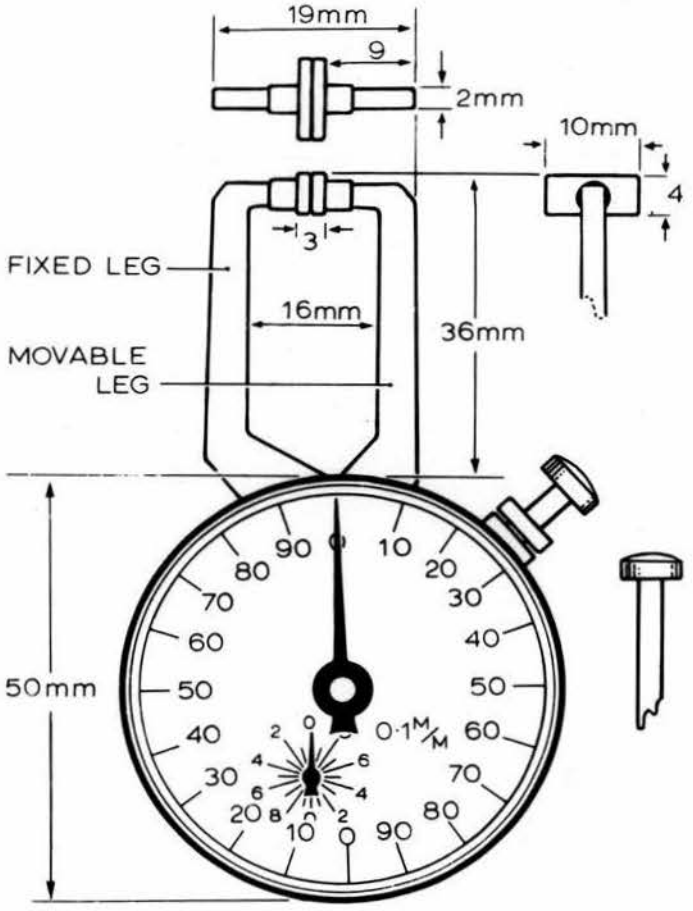
Figure 9 Minor External Caliper Gauge specially modified by British Indicators Ltd. (St. Albans, Herts.)

EXTERNAL COMPARATOR



1) Length of cranium:

measured from the external occipital protuberance to the anterior aspect in the mid-line on the nasal bones.

2) Length of braincase (calvarium):

measured from the external occipital protuberance to the junction of the internasal suture with frontonasal suture.

3) Height of face:

measured from the junction of the internasal and frontonasal sutures to the mid-point of a transverse line on the palate, joining the immediate anterior (mesial) aspect of the first maxillary molar (M^1) or, when not present, the anterior bony edge of the alveolar trough or osteodental fissure (PARK, 1969; 1973).

4) Lengths of nasal bones:

measured from the junction of the internasal suture with the frontonasal suture to the anterior aspect of the nasal bones in the mid-line.

5) Nasal bone width:

measured across the broadest width of both nasal bones over a line running at right angles to the internasal suture.

6) Least interorbital width:

measured from the upper surface of the orbital rim (superior) across the surface of the cranium to the upper rim of its counterpart nearest to it, i.e. the least breadth of the interorbital constriction as seen in dorsal view.

7) Bizygomatic breadth:

measured as the greatest breadth contained by the arches, as seen in dorsal view.

8) Width of braincase (calvarium):

measured at a point on each side of the skull sited immediately superior to the posterior roots of the zygomatic arches.

9) Biparietal width:

a measurement of the broadest width covered by the parietal bones as observed from the dorsal aspect.

10) Facial length:

measured from the anterior aspect in the mid-line on the nasal bones to the junction of the coronal and sagittal sutures.

11) Frontal bone length:

measured from the frontonasal suture in the mid-line to the junction of the coronal and sagittal sutures.

12) Cranial vault length:

measured from the junction of the coronal and sagittal sutures to the external occipital protuberance as observed from the dorsal aspect.

13) Palatal length:

measured from the posterior border of the palate in the mid-line to the most posterior aspect of the interdental septum between the upper incisors.

14) Palatal width:

measured from the lingual surface of the second maxillary molar (M^2) or, if absent, the point where the alveolar bone wall begins to curve inwards over the developing molar, to its counterpart at the other side of the skull.

15) Palatine bone length:

from the junction of the intermaxillary and maxillary-palatine suture to the posterior nasal spine.

16) Basisphenoid length:

a mid-line measurement from the centre of the spheno-occipital synchondrosis to the spheno-ethmoidal synchondrosis.

17) Basioccipital length:

from the basion to the centre point of the spheno-occipital synchondrosis.

18) Basioccipital width:

a measurement based on the biparamastoid width at its greatest.

19) Zygomatic length:

As the length measured between the most anterior point of the anterior zygomatic root and the most posterior point of the posterior zygomatic root.

20) Maxilla-premaxilla length:

as the distance between the anterior nasal spine to the posterior nasal spine.

21) Length of mandible (condyle):

the total length of the mandible measured from the posterior tip of the condylar process to the anterior tip of the interdental septum.

22) Length of mandible (angle):

the total length of the mandible measured from the posterior tip of the angular process to the anterior tip of the interdental septum.

2.14 Measurement Repeatability

Any testing of the validity of measurements of the body is hampered by the necessity to obtain records and then process the material since refrigeration was neither possible nor feasible because of dimensional tissue changes. Non-changeable measurements of the skulls, however, made tests for the standard of repeatability possible.

A pilot study was made on 30 rat skulls on the validity of their repeatability

of the measurements made in accordance with the description for each dimension, as defined in the previous section (2.13). At a later period - usually some weeks later - the skull parameters were re-measured, using identical criteria, and without reference to the original figures.

The two sets of figures were compared and since the difference between the first and second measurements was within 2% of their mean value, no further investigation was deemed necessary.

The fact that the measuring is consistent brings in the problem of skull curvature. When the total skull length was measured and matched against the added lengths of the individual bones making up that length, the slight increase in the added total proved to be within 2% of the total skull length, in other words within the accepted percentage of error. The slight difference between the total length and the added individual length depended on the exact point the measurement was made, and not only is there observer error, but the suture outlines are always irregular together with the curvature of certain parts of the skull.

The measurement of rat body-lengths was repeated by several observers at the same time and it was generally found that once the observer had mastered the technique of using a more "elastic" subject, that the amount of error was low. During this test it was noted that one person showed a higher consistency of results and stemming from this, this observer made all the whole animal measurements throughout the present work.

2.15 Biometric Analysis

Following the initial tabulation and inspection of the data obtained from both the body and cranio-facial measurements to locate possible sources of error, the means, standard deviation, standard error of mean and coefficient of variation were calculated and linear regression analysis carried out. Certain

aspects of the regression analysis will be discussed in detail later in the particular sections.

Prior to dealing with other specific matters relating to the various calculations, it is appropriate at this point to note that the terms "standard deviation" and "standard error" are used synonymously except in the present case where we are dealing with items in a sample or population. Standard error or standard deviation must be qualified by referring to a given statistic. Used without any qualification the term "standard error" conventionally implies the standard error of mean. "Standard deviation" used without qualification generally means standard deviation of items in a sample or population. Thus when the headings of means, standard deviations, standard errors and coefficients of variation appear in the text, it signifies that arithmetic means, standard deviations of items in samples, standard deviations of their means (= standard errors of means), as well as coefficients of variation are displayed. The following summarised versions of the steps taken is added for clarification:

Σnx = sum of weights or lengths etc., i.e. $x_1 + x_2 \ldots.$

Σnx = sum of squares of weights or lengths etc., i.e. $x_1^2 + x_2^2 + x_3^3 \ldots.$

n = sample number

$\bar{x}$ = $\frac{\Sigma nx}{n}$ = mean of sample

$\frac{(\Sigma nx)^2}{n}$ = $\frac{(X_1 + X_2 \ldots.)^2}{n}$

S^2 = sample variance = $\frac{(\Sigma nx^2 - \frac{(\Sigma nx)^2}{n}}{n - 1}$

S = sample standard deviation = $\sqrt{S}$

$S_{\bar{x}}$ = standard deviation of mean or standard error of mean

$$\sqrt{\frac{S^2}{n}}$$

$$C = \text{coefficient of variation} = \frac{S}{\bar{x}}$$

Analysis by means of linear regression lines will be shown in detail later in the text as part of the observations, but it is important to point out that the data stemming from both weight and length measurements over the preweaning period (birth to 20 days) and following construction of regression lines based on the daily arithmetic means, were found to differ considerably from curves fitted by eye. These curves were of various shapes although the fitting was applied to the same parameter - e.g. length which not only showed difference in shape between control and experimental litters, but also between the control litters themselves. Thus it emerged that it was not possible to construct curvilinear regression lines to the same formula for the same parameter. The logical sequence was, therefore, to break the total regression line. This was achieved either by means of the largest overall difference of the regression coefficient "b" or by selecting the mid-day in the curve where "b's" had small difference on consecutive days with an abrupt drop on either side of such a sequence. The regression coefficient "b" is basically the estimate of the slope of the rectilinear regression equation, the sample coefficient of the regression.

Because the points of total regression line "breakage" had to be calculated from every aspect, it was not possible to find these points by data programming and computerisation.

Comparison between control and experimental litters and individual animals has been achieved by the use of a number of indices:

$$\text{Ponderal Index (P.I.)} = \frac{\text{Length}}{\text{Cube root of weight}} : \frac{L}{W_3} \qquad \ldots(1)$$

$$\text{Obesity Index (O.I.)} = \frac{\text{Weight}}{\text{Length}^2} : \frac{W}{L^2} \qquad \ldots(2)$$

$$\text{Rohrer Index (R.I.)} = \frac{\text{Weight}}{\text{Length}^3} : \frac{W}{L^3} \qquad \ldots(3)$$

These three indices, as can be seen, are simply the same mathematical trick changed round to give a slightly different aspect. The Ponderal Index has often been employed by physical anthropologists for measuring and comparing weight relative to linearity of build, i.e. somatotyping. SELTZER (1966) stated that "The rationale of the index posits that weight is a measure of volume and that volume increases according to the cube of the linear dimensions". Since, as an index of shape, it excludes gross body size, it has a value for certain purposes. It is a better index of linearity and laterality than simple broad height (length) - weight categories. The ponderal index has been used in previous work and proved satisfactory within its range (PARK, 1969, 1972). The Index is generally treated on theoretical grounds as being independent of height or length. Application of the Index to human growth and size has shown some inadequacy when the range was extreme.

The Rohrer Index is basically the same as the Ponderal Index but is expressed differently. It is often known as the Index of Body Volume, or the "baric index".

The Obesity Index is probably the least useful of the three indices but has been added to complete all aspects of weight and length which can be expressed in one figure.

Although these three indices have and still are applied to the human assessment it is interesting to note the comments made by BRODY (1945) regarding the rise of weight with the linear size in geometrically similar bodies - it seems that they would be better applied to the growth and state of animals.

Within the growth period from birth to 40 days, further supplementary information can be derived from the weight. By using weight and time, the instantaneous relative growth rate "k" can be calculated. This growth rate equation was introduced by BRODY (1945) and can be expressed as follows:

$$k = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \qquad \ldots(4)$$

where k = the instantaneous relative growth rate.

InW = natural logarithm of weight W at time t.

Thus, the instantaneous relative growth rate "k" is the difference between the natural logarithm of the weights $W_2 - W_1$ divided by the time interval $t_2 - t_1$. If required, a percentage growth rate can be obtained by multiplying "k" by 100.

The constant "k" has a perfectly definite meaning and is the instantaneous relative rate of growth for a given unit in time.

Since the present investigation involves a 24-hour series from birth to 40 days, naturally the time total is short but the intervals of time $(t_2 - t_1)$ needed for the calculation of the instantaneous relative growth rate "k" must be greater than unity, for obvious mathematical considerations. To allow a complete coverage intervals of 2 days were selected and two separate series set up, i.e. odd and even day series (PARK, 1970).

A modification to the use of the instantaneous relative growth rate equation was introduced (PARK, 1969, 1970, 1971) to give a ratio comparison for examining male and female trends. The equation was changed to produce a ratio equation and thus the natural logarithms were dispensed with as follows:

Male

$$k_M = \frac{\log M_2 - \log M_1}{t_2 - t_1} \qquad \ldots(5)$$

k_M = growth rate of male.

M = weight of male.

Female

$$k_F = \frac{\log F_2 - \log F_1}{t_2 - t_1} \qquad \ldots(6)$$

k_F = growth rate of female.

F = weight of female.

By incorporating both these equations, i.e. (5) and (6), the time factor is thus eliminated leaving the following equation:

$$\frac{k_M}{k_F} = \frac{\log M_2 - \log M_1}{\log F_2 - \log F_1} \qquad \ldots(7)$$

Thus k_M/k_F is based on unity - if the result is greater than unity, then the growth rate of the male is faster than the female, if the reverse, then the female growth rate is faster. The equation is only valid if the time period is identical for both.

PART III

THE RELATION OF LITTER SIZE TO THE MATERNAL ENVIRONMENT

"Indeed, I think there is a danger that biologists may be inhibited from formulating their underlying assumptions about control relationships just because these ideas would seem to be crude if brought to light. Certainly a great deal of detailed knowledge of control relationships ..... does exist, but little of it is systemised in such a way as to provide general guides to thinking".

BASTIN, 1969.

3.1 The Significance of Litter-Size

Animals show considerable variation of both genetic and environmental origin in the patterns of their life histories. Many examples exist, for example the larger St. Kilda mouse compared to its brothers on the mainland, the albino laboratory rabbit could be regarded as another. Work by ALM (1949, 1959) on two species of trout (Salmo trutta) gives a more detailed example of variation. One form of trout (S. t. lacustris) lived in a lake environment, the conditions were good so that it grew to a large size and matured between 5-7 years. The other trout (S. t. fario) was a river dweller where the conditions were not so good resulting in a smaller size and maturation taking place at the age of 3-5 years. When these two trout were placed in the same environment, the growth rates of both became the same but the age of maturity remained different. This variation can be regarded as adaptive or the consequences of natural selection which contributes towards the fitness of the animal within the framework of its environment.

On a broad basis, considerable work has been done by a number of investigators among whom one of the first was FISHER (1958) with his analysis of the problem of sex ratio. Further attempts include those on clutch size in birds (LACK, 1954; CODY, 1966), colonising and population ability (COLE, 1954; LEWONTIN, 1965; WILLIAMS, 1966; ISTOCK, 1967; MURPHY, 1968; MacARTHUR and WILSON, 1967; GADGIL and BOSSERT, 1970).

We are dealing with a laboratory reared rat (Sprague-Dawley strain) which has obviously benefited from many generations in general laboratory conditions. With improved conditions these rats have adapted themselves and subsequently produced fairly large litters. The size of the litter should have a better chance of survival under laboratory conditions than would a litter born to its counterpart in the wild. Generally, the litter size is larger - but mainly

in the sense of survival - the young rats in a laboratory stand a better chance of weaning. In spite of improved conditions, many rat litters are too large for the mother to handle and this results in the death of a number or, stunting for some (PARK, 1964, 1968).

There is a possibility, as we have seen with the trout under similar conditions but still showing maturation time differences, that although the laboratory reared litter has increased its chances of survival due to a stable environment an increase in the maternal capacity has not been attained to the same degree. Let us presume that for theoretical purposes the female rat has increased her maternal capacity but that it is outpaced by her productive potential - this of course relative to Rattus norvegicus in the field.

The size of litter reared in the wild is not the norm for the laboratory reared rat, yet the size of litter in the laboratory is, more than often, too large for each young rat to attain his full growth potential. The question then arises - what is the most suitable average size of a litter, i.e. a control litter, in which each individual rat could achieve a full growth potential and the mother lactate at a comfortable level? Until this can be answered then comparisons with experimental litters will result in controversy.

Attempts to explain the significance of litter-size have been considered from a number of points. LACK (1948) regarded natural selection as favouring those animals which produced the most offspring and therefore leave the most descendants. To this statement he added a rider that an "upper limit is set by the number of young which the parents can successfully raise" but tempered this by agreeing that there was an evolutionary alternative between producing more young, or fewer young which were better nourished and protected. On this basic premise a number of hypotheses have emerged which view litter-size as only a part of an overall reproductive strategy but are at variance in the

parameters considered as paramount in litter-size determination. Factors influencing litter-size range from "resources", length of breeding season, body size, food supply, altitude, latitude, population stability, mortality to competition (LORD, 1960; CODY, 1966; GIBB, 1968; SMITH AND McGINNIS, 1968; SPENCER and STEINHOFF, 1968; MILLER, 1973).

Two schools of thought exist at the present time in relation to litter size, the first originated by LACK (1954) and the second by WYNNE-EDWARDS (1962). The hypothesis stemming from LACK (1954) can be summarised by accepting that litter-size is determined by the action of natural selection, i.e. the genotypes favoured by natural selection being those whose litter size eventually ended with the highest number of survivors hence the largest number of young which could be fed.

The second hypothesis concerned inter-group selection in which it was proposed by WYNNE-EDWARDS (1962) that animals within a group maintain their reproductive rate below the maximum possible rate in order to prevent the over-exploitation of the food supply.

The nucleus of contention arising from these two schools of thought can be simply stated as "the most frequent litter-size is smaller than the most efficient litter-size - the last being the size from which stems the largest number of surviving descendants". This particular aspect has been supported by work on the guinea-pig (WRIGHT and EATON, 1929), which showed that the percentage of weaned young so decreases as the litter-size increases that in litters larger than 5, the extra number at parturition was more than offset by the higher mortality between birth and weaning. For example, although the most frequent litter-size was 3 - giving rise to an average number of 2.2 descendants per litter - the most productive litter size was 5, giving an average of 2.7 weaned young per litter.

When this information was examined it appeared to be contrary to the principle of natural selection, in fact, the results might be used to support the

theory of inter-group selection because the reproductive rate was maintained at a level below the maximum.

The challenge of WRIGHT and EATON (1929) to the LACK (1954) hypothesis was refuted by the suggestion that there were three possible causes why the guinea-pig data did not support the ideal natural selection conditions:

1) That the mortality following weaning had not been brought into the calculations.
2) That laboratory environments were more favourable than natural environments.
3) That some of the litter-size differences were from adaptation, i.e. the small litter reflected the reduction of food supply.

The existence of these three factors were accepted by MOUNTFORD (1968), but not as an answer to the problem raised. He suggested that certain features of natural variability in litter-size had been overlooked. Basically, the number of young varies between the individuals of the same genotype and also between the litters produced on different occasions by the same mother. MOUNTFORD (1968) put it succinctly that the theory of natural selection did not predict that the most productive litter-size should also be the most frequent litter-size. In short, natural selection can be regarded as a statistical phenomenon which operates on all the occurrences of a genotype in a population and is concerned with the most productive litter-size (the most frequent phenotype manifestation) and the whole distribution of litter-sizes linked with the particular genotype as distinct from the distributions linked with alternate genotypes. Thus natural selection favours those genotypes that produce the largest number of surviving descendants - and this number is the sum of the separate contributions of descendants from the whole range of litter-sizes in which the most productive size is not the most frequent.

A theoretical demonstration was undertaken by MOUNTFORD (1968) in which he showed that the total number of weaned young was a combination of the frequency distribution of the different sizes of litter and the number of weaned young from each litter-size. He postulated that although natural selection favoured those genotypes that maximised this combination, there was no reason to believe that the maximum of the combination of the two combinations should coincide with the maximum of a single component such as the number of young weaned per litter. Work by MILLAR (1973) supported this contention.

Physiological variation of litter size with age can be explained by the alterations in the ovarian function with age; in some species, size and weight have a bearing in terms of availability of uterine space (JONES and KROHN, 1961). During the preweaning phase of development the limits imposed on litter-size by external environment, i.e. weather, food supplies (to the adult) etc. did not appear to be operating and it was regarded by MILLAR (1973) that the main influence was probably physiological based on factors such as energy assimilation, drainage of energy reserves and the rate of growth of the young.

The influence of the size of the litter after birth must fall mainly on the maternal capacity. In many small mammals such as bank voles (Clethrionomys glareolus) (KACZMARSKI, 1966), common voles (Microtus arvalis) (MIGULA, 1968), mice (Mus masculus) (MYRCHA et al, 1969), the red squirrel (Tamiasciurus hudsonicus) (SMITH, 1968), as well as the rat (Rattus norvegicus) both wild and laboratory species, there is an increased intake of food by the mother. In the case of the laboratory rat the problem of food supply does not arise so that the lactational problems must stem from other factors. Generally, the differential mortality in litters of different size during lactation stems from the lactational capacity of the female.

Further discussion of the factors influencing the maternal environment will be found later, but it is clear that in the case of the laboratory rat the numbers

within a litter which allows each individual member to grow to the full extent of its potential, just be found before any control litter is set up. In search of an answer, the next two experiments have been specially designed to throw some light on the subject. The extent of the experiments has been curtailed by both time and breeding facilities but sufficient data for future planning has emerged.

3.2 Experiment 1. The Influence of Numbers - Methodology

The selection of the rats depended on the arrangement of the breeding pattern so that the females would give birth within a few hours of each other. The females were all "proven" mothers having reared at least two previous litters. Once the litters were born, they were mixed together to distribute the potential genetic differences of growth before being re-allocated to a foster mother (a few young rats would be returned to their natural mother but simply by chance selection).

The litters consisted of young rats numbering from 2 to 15 per litter. In the litters with 2, three replicates were formed, while in litters consisting of 3, 4, 5 and 10 young, two replicates were formed.

Following birth, mixing and re-allocation of the young, the first 24 hours was regarded as day 1 of the series and at this point and thereafter at 24-hourly intervals, each litter was weighed en masse and the average weight of a single specimen calculated. Both total and average weight was recorded. The rationale behind the total litter weighings as opposed to single animal weighings was the reduction of the disturbance factor together with the length of time which the young would be absent from their mother, cage, the possible loss of heat, and any general introduction of stress.

After the initial tabulation of the results, the means, standard error of mean, coefficient of variation were calculated and a linear regression analysis

carried out. Calculations designed to break down the total regression line were accomplished by means of the largest overall differences of the regression coefficient "b".

3.3 Experiment 1. The Influence of Numbers - Observations

Inspection of the data tabulated in Table 1 gives an overall perspective of the average weight for a single rat representing each experimental litter. From this emerges the point that the smallest litters, which theoretically one would expect to return a greater weight, did not provide the heaviest individuals. In fact, the heaviest rats occurred in litters ranging in size from 5 to 8 rats per litter. Moreover, the results showed that in some litters basically consisting of the same number of young there was considerable variation.

Examples of variation in similar litter size were observed in litters 2a, 2b and 2c, the first two litters bore a close resemblance to each other both in size and weight, whereas the third litter was smaller and lighter. Further analysis by weighted regression lines showed that litter 2a and 2b could be regarded as similar while litter 2c should be considered as different since its overall regression line was found to be non-parallel to those of litters 2a and 2b. The significance of these differences could be attributable to either condition of the young or a defective maternal capacity.

Regression lines constructed for litters 4a and 4b, 5a and 5b, and 10a and 10b showed that there was no affinity between the pairs since their lines were not parallel. While litters 4a and 5b were provisionally designated as litters linked with a poor maternal capacity, a decision regarding litters 10a and 10b proved to be more difficult. The problem has basically one of criteria - which of each pair should be selected to form part of the series.

As a partial solution to this problem it was decided to examine the total biological weight of each litter (Fig. 10) by plotting the various litter-weights

Figure 10 Constructed to demonstrate the total biological weight of each litter and their replicates achieved by the termination of the experiment on Day 20. Note the theoretical horizontal broken line sited at the 280 *g* mark around which the weights are distributed.

WEIGHT CULMINATION AT DAY 20.

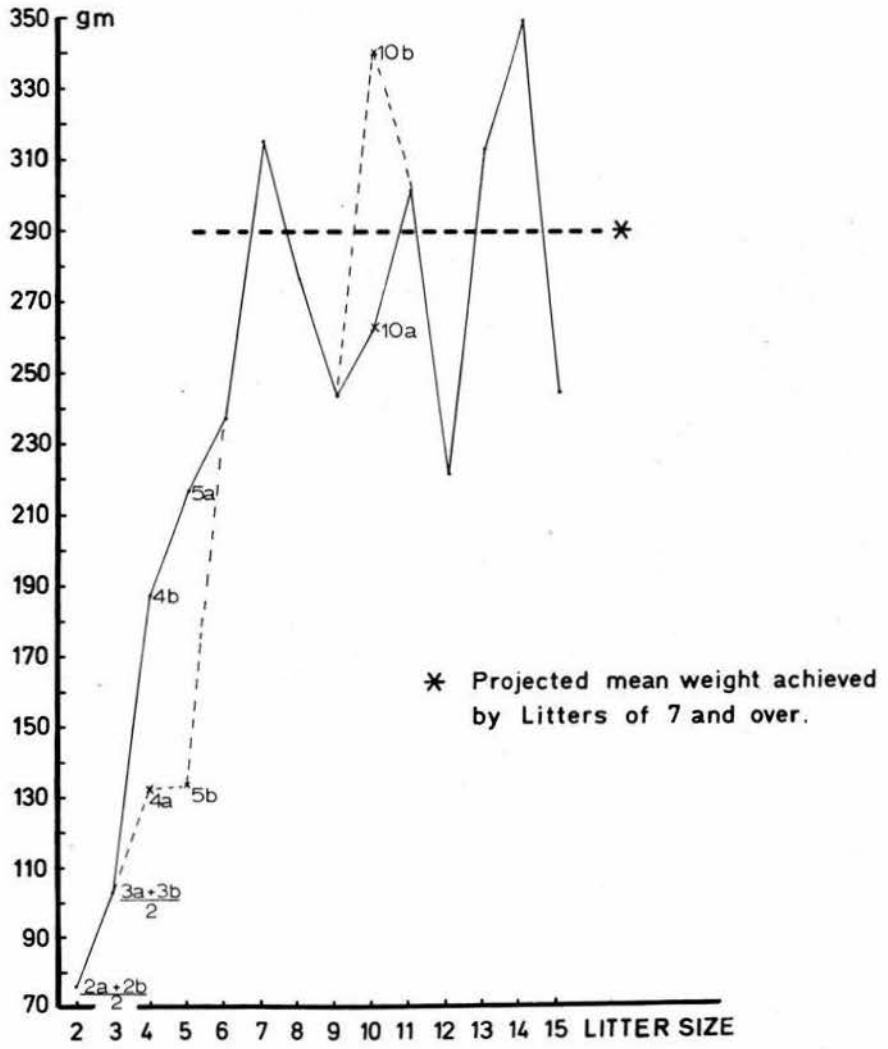


TABLE 1

AVERAGE WEIGHT OF AN INDIVIDUAL IN LITTERS OF DIFFERENT SIZE

Age in Days	Litter designation and number in litter									
	2a	2b	2c	3a	3b	4a	4b	5a	5b	6
1	6.05	6.60	6.05	5.63	5.93	6.13	6.13	8.06	6.70	6.75
2	7.25	7.70	7.20	6.10	6.40	7.05	6.53	8.44	8.16	7.20
3	8.00	9.00	7.50	7.00	7.47	8.08	8.20	9.92	9.42	8.45
4	8.50	10.15	7.00	8.17	8.70	9.35	9.55	11.26	10.78	10.82
5	11.25	11.45	7.70	10.17	10.13	10.53	11.03	13.32	12.58	12.73
6	13.50	13.40	8.70	10.97	11.40	12.13	12.83	15.08	14.62	14.18
7	15.20	15.05	9.05	12.10	12.87	14.30	14.68	16.80	16.48	16.47
8	16.50	16.25	10.45	13.53	14.33	15.35	16.80	19.12	17.80	18.98
9	18.50	18.80	11.30	15.23	16.13	16.25	18.68	21.30	18.64	20.90
10	21.35	20.45	12.25	17.77	18.73	17.40	21.53	23.04	19.02	22.87
11	23.25	22.15	12.70	20.50	21.70	18.88	25.00	25.14	20.28	25.18
12	25.10	24.00	13.65	23.50	23.33	19.63	28.48	26.88	21.34	27.83
13	26.15	26.10	14.90	25.87	26.17	22.15	31.53	28.56	21.92	29.92
14	28.15	28.30	16.10	27.90	29.03	23.03	32.88	29.82	22.44	32.60
15	30.30	29.90	16.85	31.43	32.20	24.08	36.45	34.28	23.30	34.42
16	31.20	32.40	18.25	33.37	33.80	25.55	38.43	34.52	23.82	36.07
17	33.40	34.45	19.80	34.53	35.90	27.83	39.30	35.76	24.04	37.08
18	35.15	35.95	21.60	35.50	36.53	29.58	41.13	37.62	25.56	37.35
19	35.90	37.05	22.30	36.30	37.10	31.10	43.38	37.72	25.84	36.17
20	37.60	38.20	24.55	36.90	38.03	33.10	46.55	43.24	26.60	39.48
Tot. Litter	75.80			112.40						
Wt. at day 20	75.20	76.40	49.10	110.70	114.09	132.40	186.20	216.20	133.00	236.88

TABLE 1 (Cont'd)

AVERAGE WEIGHT OF AN INDIVIDUAL IN LITTERS OF DIFFERENT SIZE

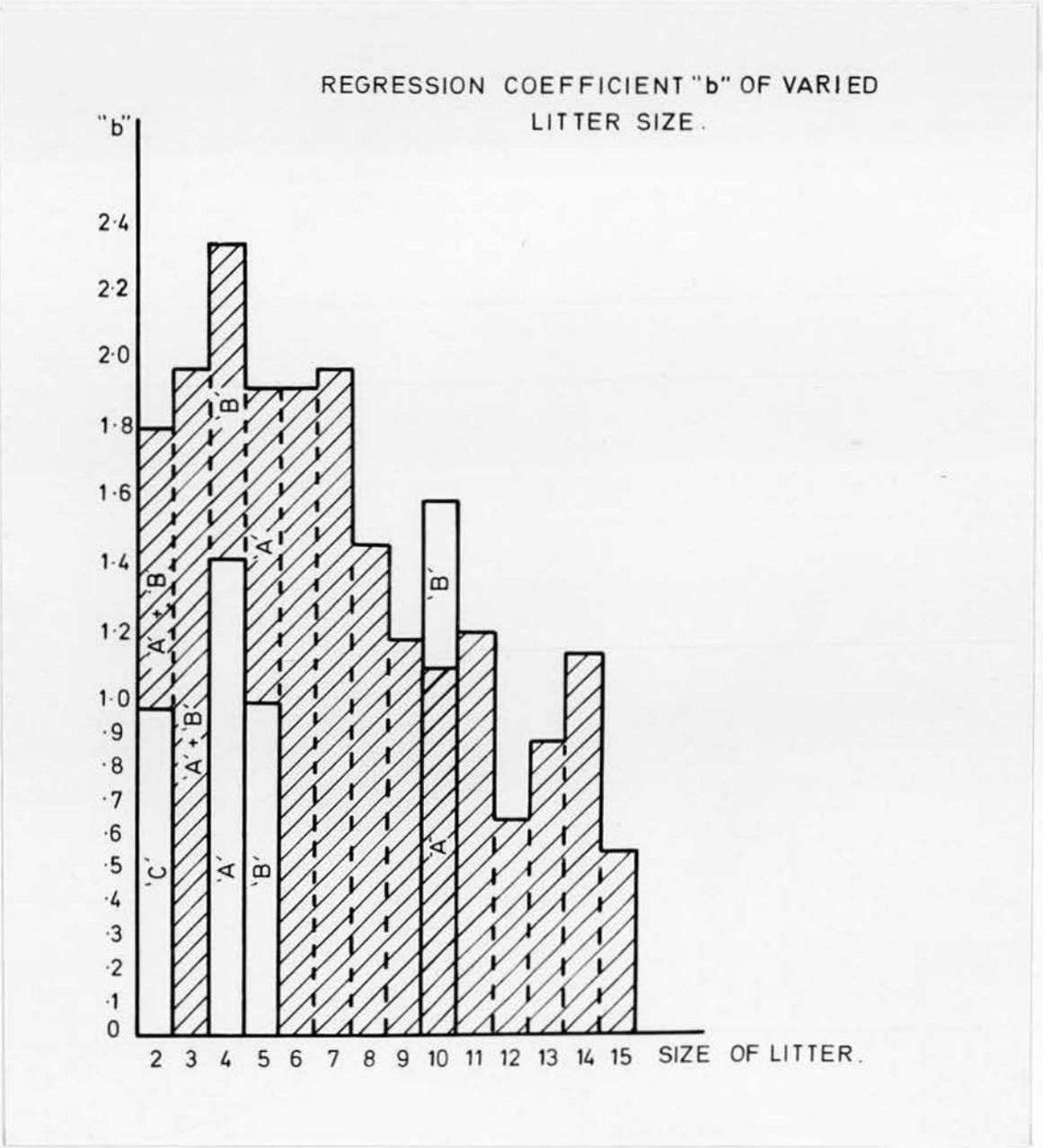
Age in Days	Litter designation and number in litter									
	7	8	9	10a	10b	11	12	13	14	15
1	7.87	6.50	5.90	6.32	5.14	5.12	6.25	6.78	5.50	5.62
2	9.16	7.58	6.44	6.90	6.44	5.77	6.67	7.73	6.35	6.17
3	11.11	9.00	7.47	8.52	7.65	6.68	7.65	8.56	7.08	7.01
4	13.36	10.70	8.71	9.48	9.13	8.01	8.49	9.83	8.29	8.41
5	15.66	12.36	10.13	11.00	10.66	9.06	9.16	10.76	9.16	8.95
6	17.36	14.60	11.44	12.02	12.14	10.25	9.98	12.22	8.99	9.61
7	19.11	16.31	12.49	13.40	13.37	11.49	10.43	13.22	11.69	10.37
8	21.57	18.23	14.36	14.81	16.00	12.69	12.34	14.08	9.86	11.09
9	23.31	19.91	15.56	15.75	17.50	13.86	12.85	15.09	14.01	11.57
10	25.94	21.19	16.19	17.24	19.20	15.21	13.21	16.58	15.36	12.02
11	28.99	22.78	17.14	18.16	20.84	16.96	14.38	16.77	16.18	12.68
12	30.19	24.30	18.61	19.08	22.30	18.01	15.27	17.11	17.11	13.24
13	32.29	26.03	20.14	20.08	24.61	19.02	15.61	18.22	19.09	13.80
14	33.94	28.20	21.46	21.37	25.86	20.22	15.61	18.83	20.41	14.31
15	85.36	29.80	22.63	21.71	27.13	21.07	16.00	19.50	21.21	14.67
16	36.60	30.38	23.48	23.64	28.78	21.96	16.18	20.38	22.32	14.91
17	38.24	30.45	24.33	24.68	30.10	23.22	16.73	21.10	23.05	15.11
18	39.76	30.01	25.24	25.20	31.23	24.48	17.12	21.91	24.07	15.46
19	42.60	29.10	25.82	25.44	32.27	25.44	17.45	22.76	24.23	15.84
20	44.91	34.51	27.00	26.25	34.00	27.42	18.45	24.11	24.94	16.32
Tot. Litter Wt. at day 20	314.37	276.08	243.00	262.50	340.00	301.62	221.40	313.43	349.16	244.80

achieved by the termination of the series on the 20th day. In totality, the graph indicated that with the increase from 2 to 6 or 7, there was a regular linear increase in the total litter-weight and hence it is possible to postulate a theoretical horizontal line situated in line with the 280 gram mark about which the weights were distributed. Due to laboratory conditions it was not possible to form replicate litters of the larger litters, thus a meaningful regression line could not be constructed.

The next step in the investigation was to examine the slope of the individual regression lines (Fig. 11) and this was presented in the form of a histogram. The outline clearly showed the general trend with the slopes increasing from litters of 2 to 4, thereafter decreasing. Litters regarded as being under the influence of poor maternal capacity - 2c, 4a and 5b - were shown for comparison, while litter 10b was finally accepted as a litter with a better maternal environment than litter 10a. Figure 11, in conjunction with the data presented in Figure 10 and Table I, suggests that litters of 11 and 14 appear to be above average in performance, i.e. a good maternal environment, whereas the litter of 12 appears to indicate the reverse situation - a poor maternal environment.

Selected and combined litters as well as those without replicates, had their regression lines broken at day 5 and from this point new regression lines were constructed from day 1-5. The data for days 5-20 was approached in a similar manner, but only after the point of the second break had been determined. The criteria related to this regression line break occurring at day 5 has been discussed in detail in previous work (PARK, 1972; PARK AND NOWOSIELSKI-SLEPOWRON, 1971, 1972a, b) where it was found applicable to the albine laboratory rat (Rattus norvegicus) and the rice rat (Oryzomys palustris natator) for weight. Originally, the reasons suggested leading to the point of the second break in the regression line of the preweaning stage of development

Figure 11 This demonstrates the slope of the individual regression lines calculated on the break-down of the total regression lines by means of the largest overall differences of the regression coefficient "b". Letters refer to replicate litters while unshaded columns of 2C, 4A and 5B represent poor maternal capacity. Column 10B represents an above normal maternal capacity.

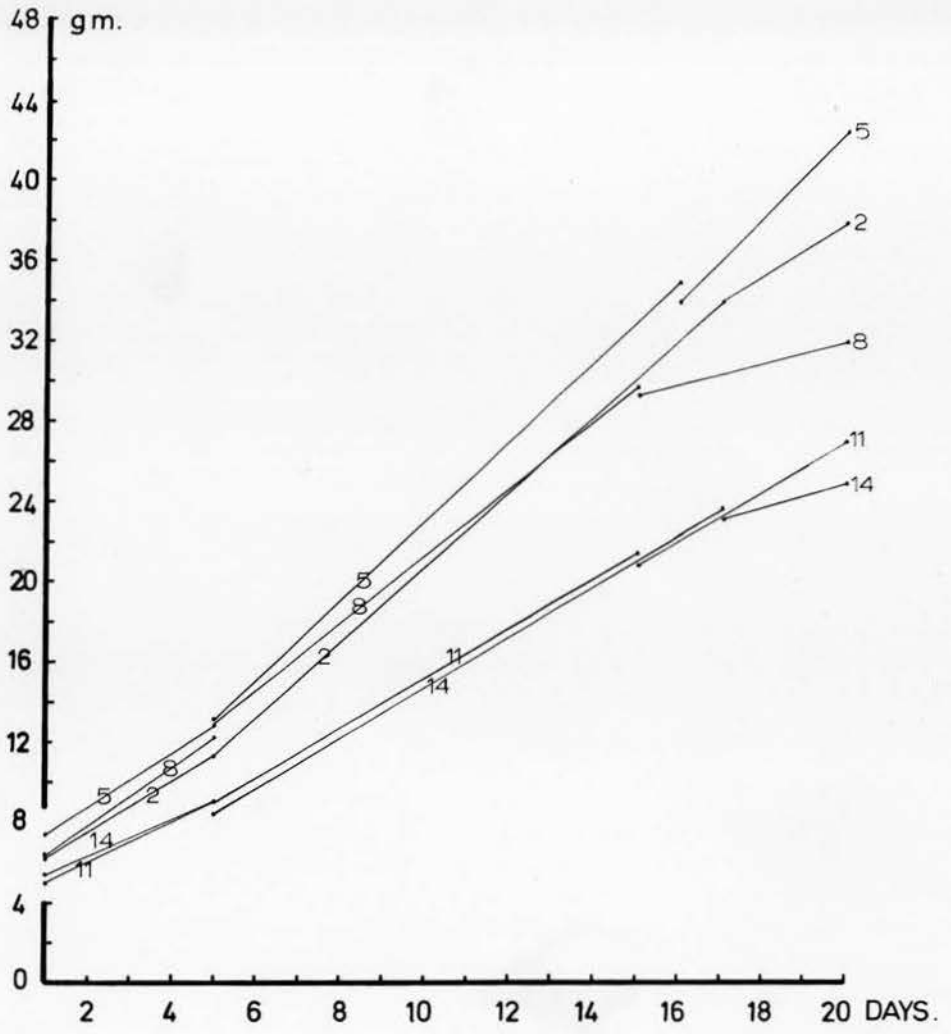

REGRESSION COEFFICIENT "b" OF VARIED LITTER SIZE.
"b"
2·4
2·2
2·0
1·8
1·6
1·4
1·2
1·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
0
'C'
'A'+'B'
'A'
'B'
'A'+'B'
'A'
'B'
'A'
'B'
2 3 4 5 6 7 8 9 10 11 12 13 14 15 SIZE OF LITTER.

were based on the greatest overall difference, the divided lines showed in the regression coefficient "b". In the light of present circumstances, however, the conclusion has been reached that in certain instances where the difference b_1-b_2 changes sign - a phenomenon commonly occurring in large litters, but never in small - that this should be regarded as the day of the second break. Basically this particular break indicates the outward sign of growth by increased weight achieved by means of food supplies from sources other than the milk of the mother.

The regression coefficient calculated for all the regression lines is shown in Table 2 with the last four columns indicating the differences of b_1-b_2 and the selection of the second break in the line. The second breaking points were tentatively established in previous work (PARK and NOWOSIELSKI-SLEPOWRON, 1971) and these were not found to deviate substantially from the present observations although there are no doubts that the breaks are not consistent. This can be shown when the second break is selected by either of the foregoing criteria and produce the following breakage points:
litter of 2 - day 17, litter of 3 - day 17, litter of 4 - day 15, litter of 5 - day 16, litter of 6 - day 16, litter of 7 - day 16, litter of 8 - day 15, litter of 9 - day 16, litter of 10 - day 17, litter of 11 - day 15, litter of 12 - day 14, litter of 13 - day 15, litter of 14 - day 17 and litter of 15 - day 14.
The general trend indicated by these results shows that the larger litter can be correlated with the earlier extra-maternal feeding.

Regression line breaks for litters of different sizes (Figs. 12, 13, 14) show that the weight differences had emerged as early as day 1. Some of the lines for days 1-5 in both position and angle of slope. In the later days regression lines for days 5-14, -15, -16 or -17 showed a certain degree of divergence, as also did similar lines for days 14-20, 15-, 16- or 17-. It was not

Figure 12 Regression line analysis showing gain in weight calculated for litters consisting of 2, 5, 8, 11 and 14 young respectively based on the means.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11
14

Figure 13 Regression line analysis showing gain in weight calculated for litters consisting of 3, 6, 9, 12 and 15 young respectively based on the means.

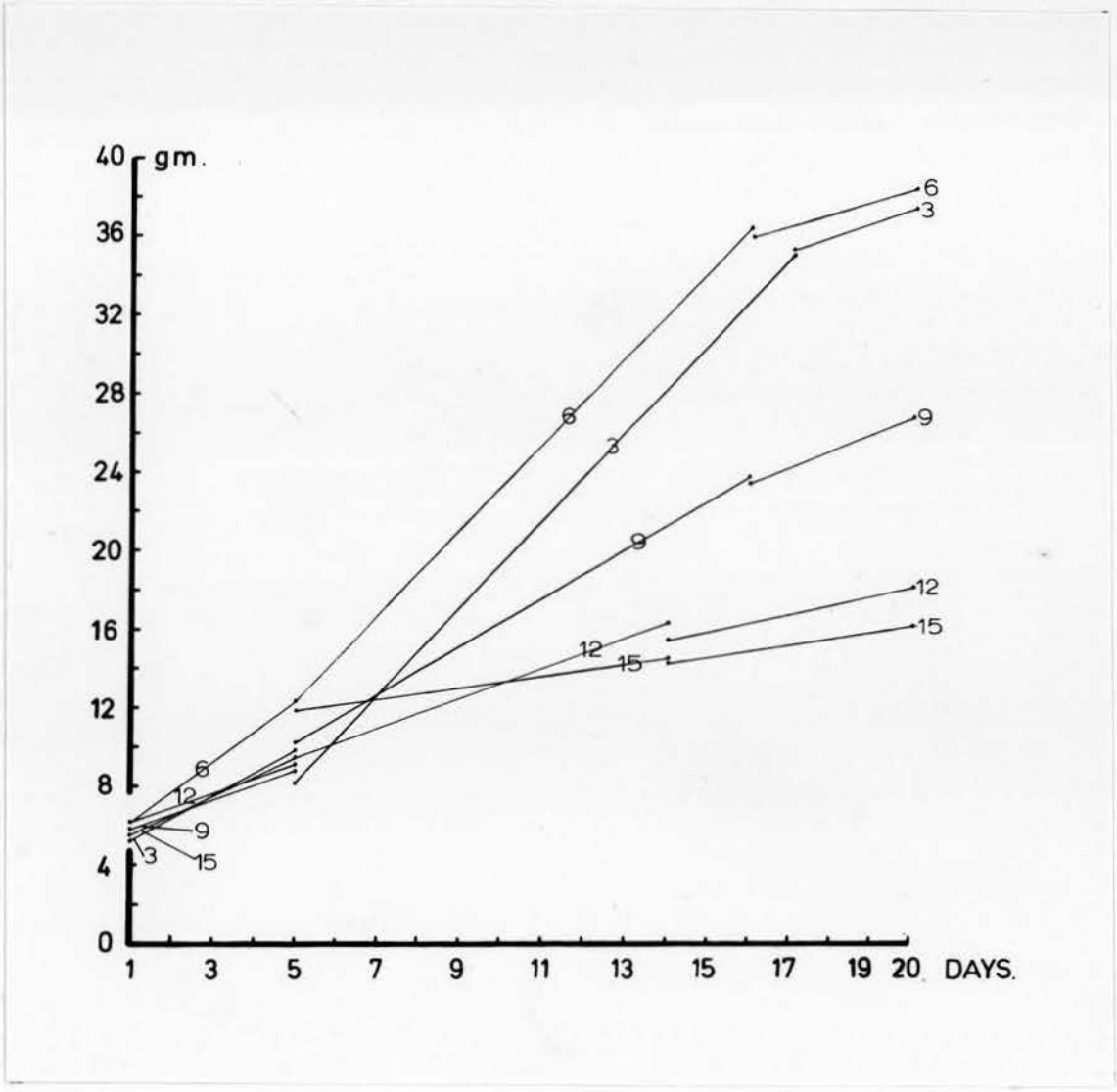
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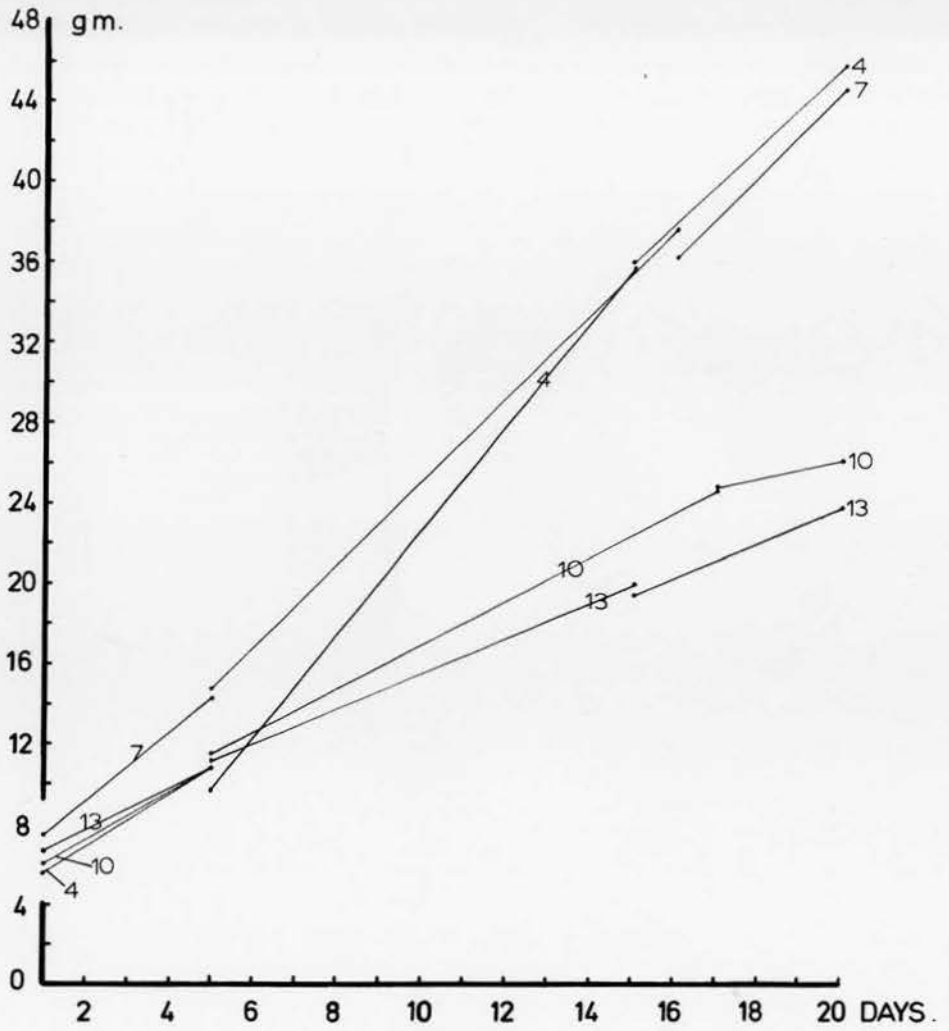
Figure 14 Regression line analysis showing gain in weight calculated for litters consisting of 4, 7, 10 and 13 young respectively based on the means.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4

TABLE 2

SAMPLE REGRESSION COEFFICIENTS "b" FOR LITTERS OF DIFFERENT SIZES

Litter	Regression Line Analysis at Selected Intervals (Days)									
Number	1 - 20	1 - 5	5 - 14	5 - 15	5 - 16	5 - 17	14 - 20	15 - 20	16 - 20	17 - 20
2a + b	1.7883	1.2390		1.8805	1.8732	1.8773		1.6148	1.5990	1.2840
3a + b	1.9637	1.0920		2.2048	2.2484	2.2486		1.0974	0.9260	0.7429
4b	2.3303	1.2820	2.5696	2.6139	2.6150	2.5581	2.0560	1.9194	2.0320	2.4000
5a	1.9094	1.3340		1.9865	1.9708	1.9366		1.7854	2.1400	2.4540
6	1.9109	1.5580		2.2224	2.2014	2.1495		0.7608	0.5910	0.6020
7	1.9552	1.9776		2.0656	2.0045	1.9556		1.9226	2.0980	2.2851
8	1.4474	1.4840	1.6815	1.6888	1.6504	1.5751	0.6129	0.5506	0.6910	1.1270
9	1.1699	1.0730	1.2269	0.9488	1.2228	1.2033	0.8814	0.8452	0.8530	0.8590
10a	1.0932	1.1940	1.1469	1.1069	1.1164	1.1187	0.8450	0.8177	0.5980	0.4950
11	1.1891	1.0120	1.2641	1.2387	1.2103	1.1052	1.1735	1.2414	1.3140	1.3560
12	0.6332	0.7640	0.7667	0.6865	0.6728	0.6378	0.4414	0.4699	0.5260	0.5489
13	0.8677	1.0060	0.8705	0.8443	0.8306	0.8186	0.8535	0.8857	0.9120	0.9880
14	1.1344	0.9260	1.3294	1.3163	1.3008	1.2726	0.7432	0.7256	0.6420	0.5829
15	0.5392	0.8910	0.5887	0.5736	0.5520	0.5274	0.3185	0.3254	0.3550	0.4009

TABLE 2 (Cont'd)

SAMPLE REGRESSION COEFFICIENTS "b" FOR LITTERS OF DIFFERENT SIZES

Litter	$b_1 - b_2$ in period day 5 - 20 at selected days			
Number	14	15	16	17
2a + b		-0.2657	-0.2742	-0.5933*
3a + b		-1.1074	-1.3224	1.5057*
4b	-0.5136	-0.6944*	-0.5830	-0.1581
5a		-0.2011	+0.1692*	+0.5174
6		-1.4616	-1.6104*	-1.5465
7		-0.1430	+0.0935*	+0.3295
8	-1.0686	-1.1374*	-0.9594	-0.4481
9	-0.3455	-0.1036	-0.3698*	-0.3443
10a	-0.3046	-0.2892	-0.5184	-0.6237*
11	-0.0906	+0.0027*	+0.1037	+0.1608
12	-0.3253*	-0.2166	-0.1468	-0.0889
13	-0.0173	+0.0414*	+0.0814	+0.1694
14	-0.5862	-0.5907	-0.6588	-0.6897*
15	-0.2702*	-0.2482	-0.1970	-0.1265

* Selected breakage point of regression line.

regarded as meaningful, at this point, to make a detailed analysis of these lines, but rather to examine their relative position to one another. Thus it will be observed that the litters of a large size did not grow as well - weight-wise - as the smaller litters, and as an adjunct it was noted that very small litters when equated to medium sized litters were found to be inferior. In short, a litter of "2" did not equal the response that a litter of "5" achieved; a litter of "3" was also poorer compared to a litter of "6", while a litter of "4" proved to be similar in its rate of progress as a litter of "7".

For elucidation of the effects of different litter-size, Table 3 was constructed to give perspective and support the indications that small litters consisting of 4, 5, 6 or 7 individuals were found to gain weight more rapidly than the much smaller litters of 2 or 3 individuals.

Refutation of this finding, however, emerged on examination of Figure 10 where the total biological gain introduced some doubts as to the exact status of a litter composed of 4 individuals. On the other hand, Figure 11 demonstrates that with a litter of 4, the overall (1-20) regression line indicates a rapid weight gain since the regression coefficient "b" was the largest. Basically, on the evidence available, it must be accepted that a litter of 4 is a member of the optimum litters. Medium sized litters of 8, 9 and 10 were not found to grow so well as smaller litters, but had a definite weight increase relative to litters composed of 11, 12 or 13 individuals.

The inference underlying the varying growth performances can be linked with the lack of sufficient numbers of young rats in small litters to either stimulate or maintain lactation. The converse of this can be inferred with the large litters where stimulation must have reached its maximum but the finite milk supply is being shared between many more mouths.

TABLE 3

MEAN WEIGHT OF INDIVIDUALS IN LITTERS OF VARIOUS SIZES

Size of Litter	Wt. at Day 1	5	10	15	20
2a	6.2200	11.1760	20.7073	30.0938	37.8910
3	5.3840	9.7520	19.3898	30.6328	37.4668
4	5.7240	10.8520	22.7172	35.7867	45.6718
5a	7.5320	12.8680	22.9929	32.8469	42.4520
6	6.0740	12.3060	23.2451	34.2521	38.4120
7	7.4768	15.3872	25.6928	35.7152	44.6180
8a	6.2600	12.1960	21.2464	29.6904	32.0848
9	5.5840	9.8760	15.7497	22.4751	26.8800
10	6.0560	10.8320	16.7998	22.3933	26.1350
11a	4.9340	8.9820	15.2582	21.4517	27.0351
12	6.1160	9.1720	13.2673	15.9086	18.1156
13	6.7200	10.7440	15.6709	19.8924	23.8408
14a	5.4240	9.1280	14.7604	21.1234	24.9468
15	5.4500	9.0140	12.0583	14.5944	16.1869

Figures calculated from regression lines:

1 - 5 from First Regression Line
10 from Second Regression Line
15 from Second or, when not available, from Third Regression Line
20 from Third Regression Line

3.4 Experiment 1. The Influence of Numbers - Discussion

Maternal effects inevitably introduce important complications to any study of animal growth, especially when linked with inheritance since they can be a major source of resemblance between maternal relatives, and their effects may also screen or confuse with resemblance resulting from genetic similarities between those relatives. This is the central source from which quantitative genetic theory stems, and to accomplish any further advances, a greater understanding of the nature and magnitude of maternal influence has become paramount.

The term "maternal influence" or "environment" can also be construed as "behavioural" when dealing with the physical (emotional) interactions of a mother and her offspring. Thus we have two definite groups or spheres of influence, namely the maternal environment, i.e. the maintenance of nutrition, nursing, etc., and the genetic endowment which stems from both parents. To these two spheres must be added a third, that of "external environment", i.e. factors outside the control of the maternal environment and the genetic endowment. The variables implicit in the concept of these controlling factors are represented in the present experiment by those of "nutrition". The control exerted on the growth process by nutrition has been demonstrated by experimentation of litter size.

Probably the best known and widely studied characteristics of young rodents reared in different sizes of litters are their differential weight or length growth rates (HAIN, 1935; SCHULTZE, 1954; SEITZ, 1954; KENNEDY, 1957; WIDDOWSON and KENNEDY, 1962; KOWALEWSKI, 1965; IRVINE and TIMIRAS, 1966; KUMARESAN et al, 1967; PARK, 1968, 1969; PRIESTNALL, 1970). As a general consensus from the observations made by various workers, it can be concluded that animals suckled in large litters receive less nourishment than those suckled in small litters and the weight was found to be negatively correlated with the size of the litter.

The general preweaning growth of a rat from any size of litter, can be regarded in the initial stages as an index of the nutritional quality and quantity of milk supplied by the mother. By reducing the size of the litter, the variation in the maternal environment was eliminated as far as possible to allow an adequate milk supply to be available (PARK, 1968, 1969; PARK and NOWOSIELSKI-SLEPOWRON, 1971, 1972). As a direct result, variation in the growth of the whole animal was minimal, a point which also served to indicate that the influence of the maternal environment was a predominant controlling factor. The converse of this reaction was obtained when large litters were used, i.e. a much wider growth variation was found stemming from an unequal division of an inadequate milk supply.

Reduction of the number of young in a litter has resulted in attaining maximum growth (GATES, 1924; PARKES, 1926; McDOWELL et al, 1930; BRODY, 1942). Various methods were tried from a direct drop of the numbers in a litter to gradual removals. There appeared to be a lower limit whereby a very small number in the litter failed to stimulate the mother to lactate. KENNEDY (1957) and WIDDOWSON and McCANCE (1960) obtained the maximum differences of growth by using small litters consisting of three rats each as compared with large litters composed of 15-18 rats. The fact that the weight of the rats at birth were connected with the size of the litter was noted by McCANCE (1962a) - a problem solved partially by pooling all the rats at birth, mixing, and then redistributing by random selection to the various mothers. At the end of the weaning phase the young rat's size tended to vary inversely with the number of litter-mates in competition for the food supply.

During a previous pilot study (PARK and NOWOSIELSKI-SLEPOWRON, 1971, 1972) the number of rats assigned to the so-called small litter was maintained throughout at 6, while the larger litters contained numbers varying from 13-18. The

variable state of the large litters was basically due to litter mortality over which no control was possible. Re-examination of the original raw data from these studies - mainly for weight - showed that many of the larger litters which had undergone some loss of numbers by mortality had actually achieved a greater weight than expected from the plot, while the very large litters which still retained their full initial complement of young, were lighter in weight.

The main difficulty arising from these findings was that this raw data had been originally obtained from a series of litters killed at selected intervals and not sequential sampling. In order to gain a correct perspective the present experiment was formulated in such a way that litters of various sizes have been weighed at selected intervals ranging from birth to 20 days thus producing a series of sequential samples pertaining to a sequential growth rate.

The salient features which have emerged from the literature concerning the variable size of litters in natural conditions make us aware of the need to standardise the number of young used for experimental purposes before differences in litter-size become translated into differences of growth.

Size of rodent litters can be directly related to the age of the female (ROMAN and STRONG, 1960; FINN, 1963; RUGH and WOHLFROMM, 1967), the type of strain used (RODERICK and SCHLAGER, 1966; FESTING, 1968), the amound of inbreeding (RODERICK and SCHLAGER, 1966), female parity (BIGGERS, FINN and McLAREN, 1963; RUGH and WOHLFROMM, 1967), and also the breeding season (BATTEN and BERRY, 1967).

Females producing litters of different sizes may have a different genotype (PRIESTNALL, 1970) and there are a number of physiological factors within the gestation period which affect numbers and subsequent early survival. The

initial weight of a litter at birth depends upon the size of the litter and this in turn has been linked with the duration of gestation (McCANCE, 1962a), this apparently being shorter for large litters (McCANCE, 1962a; McLAREN and MITCHIE, 1963) and regarded as due to the amount of distension which the uterus could tolerate.

In 1935 DAGGS stated "It is of primary importance to limit litters to a definite number at birth". Previously (DONALDSON, 1925) it had been pointed out that relatively small litters grew faster than large litters and it is presumed that these larger litters eventually followed BERTALANFFY's principle of equilfinality and achieved the accepted species-size. The size of the rat at weaning was regarded by WIDDOWSON and McCANCE (1960) and McCANCE (1962a) as tending to vary inversely with the number of litter-mates competing for the same food supply.

The present observations cover a period of growth which can be loosely termed the "preweaning" period although in actuality it basically consists of a triphasic growth pattern under-pinned by the types of food supply which can be classified into 1) milk, 2) milk and solid food, and 3) solid food only (PARK, 1969, 1972). The results of these different types - which also vary in quantity, quality and availability, i.e. even distribution - are observed by the alteration of the weight changes. Other factors operating on the food supply are those of the behavioural type, i.e. maternal behaviour. This depends on such aspects as the experiences undergone during the female's own development (RIESS, 1954; EIBL-EIBESFELDT, 1955, 1956; BENIEST-NOIROT, 1958; WEHMER, 1965), the experiences undergone during pregnancy (LEVINE and KING, 1965; DENENBERG, 1966), and the increase of maternal expertise which is the reward of practice.

The effect of the presence of young on the female's maternal behaviour

and physiological reactions also holds an important role. For example, the younger the offspring the greater the stimulation to the mother (NOIROT, 1964). Studies of rodent families have shown that the maternal response, i.e. retrieving the young, begins to decline around the 11th to 13th day period. This particular time coincides with the full use of the eyes and the ceasing of ultrasonic cries (ZIPPELIUS and SCHEIDT, 1956; NOIROT, 1966a, b).

From the observations it is clearly demonstrated that the size of a litter plays an important part in the size (for the purpose of this experiment - weight) of the rat, and that four general divisions of litter-size can be isolated in terms of weight increase relative to each group. These groups consist of very small litters (1-3), small litters (4-7), medium litters (8-10), and large litters (11-13). There is evidence in rats that milk yield is related to litter-size, (EDWARDSON and EAYRES, 1967; KUMARESAN et al, 1967; MOON, 1969; MORAG, 1970) and it has been shown that the availability of milk depends upon the functioning of the "milk-ejection reflex" (CROSS and HARRIS, 1952). Stimulation of the nipples appears to be an important factor in milk secretion. The female albino rat usually has 6 pairs of nipples - 3 in the pectoral area and 3 in the pelvic region. Work by WEICHERT (1942) showed that there was some correlation between the size of a litter and the specific nipples in use, and it was shown that selection of nipples followed an anterior-posterior sequence.

Naturally, a large litter would incite a greater stimulation to more nipples than a small litter (KUMARESON et al, 1967) and hence more milk. However, once a litter has reached a number greater than the number of nipples present (note that this does not take into account the number of nipples which are non- or only partially functional), then the quantity of milk-source available for each rat falls (SCHULTZE, 1954; KUMARESON et al, 1967; OTA and YOKOYAMA, 1967).

The less dramatic weight increases of the very small litters (1-3) compared

with the small litters (4-7), can be regarded as due to a lack of stimulation of the mother. These small litters, in comparison with medium-sized litters (8-10) and large litters (11-13), have obviously been able to obtain enough milk to allow the growth to continue at its maximum potential. In the case of the medium and large litters, the increase in size and therefore a greater number of rats competing with each other for food, has had the effect of slowing the weight gain.

Underlying the problems of the size of the litter, the degree of stimulation and the amount of milk supplied, is the triphasic growth pattern covering three nutritional phases (PARK, 1972; PARK and NOWOSIELSKI-SLEPOWRON, 1971, 1972a, b). Irrespective of the size of the litter and variation of the food source and quantity, the growth of the young rats continued to demonstrate three phases, each exhibiting its own individual growth rate by means of the slope of the regression line. The fact that each individual litter grew at its own rate and still formulated a distinctive triphasic pattern of growth, appears to indicate that the general growth potential was not as sensitive to food changes as much as that of weight. Work by REDDY and DONKER (1964), while investigating lactation, came to similar conclusions. Litter growth is therefore constant although at different rates. Leaving maintenance of the animal to one side, if the growth efficiency remained constant and the growth rate was constant, then the supply of food must be increasing throughout the observed period of 20 days. This is based on the premise that maintenance needs to be increased along with weight gain. Thus the first phase (day 0-5) is covered by a rising milk production, the second phase (day 5-14) consists of the rise of milk supplies to a maximum peak with the start of solid food intake - which must be more than just that required to replace the loss of nutrition as the milk declines since it must continue to support growth efficiency and maintenance, and the third phase (day 14-20) where solid food supplies have no specific limits.

To achieve the same daily gain of weight each day more food is going to be required each day and it is obvious that although we have used litter weight as a method of measurement, the relationship between the weight gain of the young throughout the first 20 days of development, and the food supply from the mother and other sources, is complex to say the least and can only therefore be expressed as a trend when applied to the weights of the young.

Insofar as the present experiment has gone, it can be concluded that for a steady growth performance with as little fluctuation in size as possible throughout the first 20 days of postnatal life, that litters between 4-7 young in number should be used, thus the more rounded and popular figure of 6 should produce good results. The problem of maximum lactational stimulation is one linked to larger numbers in the litters and if these are maintained the demand overcomes the supply with resulting uneven distribution. The next experiment will deal specifically with this problem where it is hoped to demonstrate the continued effectiveness of greater functioning of the "milk-ejection response" as applied to small litters.

3.5 Experiment 1. The Influence of Numbers - Summary

Uniformity of growth of laboratory animals is of direct interest to any animal experimenter and the causes of variation depend on the genetic endowment, environmental factors with persistent effects, and environmental factors with transient effects. In the environmental field, maternal effects introduce important changes in the growth potential of young suckling rats as demonstrated by weight and length.

The present work deals with the "standardisation" of the growth potential of rats during the preweaning period, the variance of which appears to be mainly attributable to the phenotype and experience of the mother. A postulate indicated (FALCONER, 1967) that the 12-day weight of a standardised litter of mice might reflect the amount of milk available, and it was found later that 60% of

variation in individual 12-day weight was due to the maternal influence (EL OKSH et al, 1967). Recently it was shown (EISEN et al, 1970; EISEN and HANRANAN, 1970) that much of the maternal variation was due to environmental or non-additive genetic effects.

Any move towards uniformity is an important asset and thus the question arises of what is the best size of litter which an average rat mother can raise which allows the expression of the fullest growth potential. The influence of litter-size was investigated by means of albino rats (Sprague-Dawley strain). The breeding females for the experiment were all "proven" mothers and their litters were arranged so that birth occurred within a few hours of each other. At birth the young were mixed to reduce any potential genetic differences and were distributed by random selection to the mothers - the cross-fostering technique. Litters consisted of rats numbering from 2 to 15 per litter. The first 24 hours after birth was regarded as day 1, and at this point and thereafter at 24-hourly intervals, the litters were weighed en masse and the mean weight of a single specimen calculated. The results were processed and a linear regression analysis carried out.

Body growth by weight and length during the preweaning phase of development (birth to day 20) has been shown to be triphasic. This consisted of breaks in the regression lines occurring at day 5 and day 15. The first phase (0-5) covers the rising milk supply, the second phase (5-15) covers the peak of milk supply and the waning, while the third phase involves the intake of solid food. The litter-sizes in the experiment reflected these phases and showed the extremes of the lactational capacity.

Litters consisting of 2-3 young did not achieve their full potential growth rate as compared to litters of 4-7. Litters ranging from 8-10 did not grow well while litters of larger numbers of 11-15 showed signs of retardation.

From these observations, it appears that the external stimulatory factors of lactation are insufficient from very small litters, while giving a maximum level to the large litters. The increased lactational capacity of the mothers of large litters is nullified by the greater demands and uneven distribution to the young. Those litters containing 5 or 6 young rats appear by weight to be on or near the point which satisfies the growth potential.

3.6 Experiment II. Maternal Capacity and Stimulation - Methodology

Stemming from the deductions arising from Experiment I and information gleaned from literature of many disciplines, Experiment II has been designed whereby the "mother" rats would receive a near maximum initial stimulation with a large litter followed by a reduction to a smaller size. The basis for such a procedure has arisen from the observations in Experiment 1 where the reduction of litter-size by the natural process of infant mortality enabled the remaining young to increase their milk intake thus stimulating the growth rate and giving a rapid increase in weight.

The experimental litters originated from 18 female laboratory rats (Sprague-Dawley strain) which were selected on the merits of their breeding capacity and maternal behaviour. The majority of these rats had successfully completed two pregnancies and reared both litters until the weaning point had been reached. A number of the rats had reared three litters. In all instances the overall maternal influence was graded as good - all other mothers not reaching this standard having been removed from the list.

Breeding was arranged to enable a number of litters to be born on the same day (see Part II - Materials and Methods) and very often within a few hours of each other. These litters were then mixed together to distribute as evenly as possible any potential genetic differences in growth before re-allocating them in litters numbering 14 rats each to the experimental foster mothers.

Each experimental litter was designated with a code letter ranging from 'A' to 'R' while the control litter was recruited from Experiment 1 - a litter of 14 - and this was given the code letter 'S'.

Following the litter formation, each was weighed at daily intervals starting with the first day following birth. Weighings were conducted under identical conditions of handling and always took place at the same time each day. Laboratory conditions throughout the experiment were maintained with a temperature range of 23-25°C and a relative humidity of approximately 50%.

Throughout the period of observation of the litters from birth to 20 days, a sequence of changes in the number of rats contained in each litter were made at three definite stages. These stages were initiated at 5, 10 and 15 days after birth.

Change on day 5: Litters coded A to E were reduced in numbers as follows - A to 4, B to 6, C to 8, D to 10 and E to 12.

Change on day 10: Litters coded F to K were reduced in numbers as follows - F to 4, G to 6, H to 8, I to 10, J to 12. A further litter coded as K was reduced to 12 to form a replicate of J.

Change on day 15: Litters coded L to R were reduced in numbers as follows - L and M to 4, N to 6, O to 8, P to 10 and Q and R to 12.

Biometric analysis was undertaken and initially two regression lines were calculated for each litter. These consisted of a regression line construction from birth to the day of reduction, and then from the day of reduction to day 20 - the day on which the experiment terminated. Later four linear regression lines were constructed for each litter based on phases Day 1 to 5, 5 to 10, 10 to 15 and 15 to 20. These calculations allowed insight into the amount and rate of growth by weight.

3.7 Experiment II. Maternal Capacity and Stimulation - Observations

A spectrum of the changes occurring to the average weight of an individual rat from various litters is demonstrated in Figure 15. From this it can be seen that the initial weight obtained on day 1 shows a considerable variation ranging from 5.26 g - 6.97 g at a period when all the litters contained 14 young. This shows that the individual rats - which were originally mixed to distribute any possible potential genetic differences in growth - had been selected at random with no attempt to even the litter weights.

The variation in difference of weight is found to continue into the day 5 phase during which the total number of individuals were retained at 14. The uneven weight continued to persist so that the heavier litters maintained their dominance over the lighter litters - note litters H and O, and conversely B, F and Q.

On day 5 the numbers of rats were reduced in the litters coded A to E: A - 4, B - 6, C - 8, D - 10 and E - 12 (Figure 22). The removal of the surplus animals was made by random selection.

The effects of these removals became evident by day 10 as shown by litters A - 4, B - 6 and C - 8 which had all increased their overall weight compared to litters D - 10 and E - 12. In fact, the weight increases had been accomplished at a greater rate than the other litters - F to S - which had retained their original number of 14 young during the period of 5 to 10 days. A considerable variability existed in litters F to S, ranging between 12.97 g for Q and 17.01 g for P, which reinforces the postulate that litters of the same size do not always grow at the same rate due to a probable combination of lactational capacity, general maternal behaviour and a small amount of genetic influence.

The variation can be shown by the coefficients of the regression "b" (Table 4) and it was found that the experimental replicates of J and K, L and M

Figure 15 Histogram demonstrating the average individual weight relative to the sizes of the litters in relation to time - birth, 5, 10 and 15 days. The control column coded S consisting of 14 rats is derived from Experiment I (Section 3.3. to 3.5).

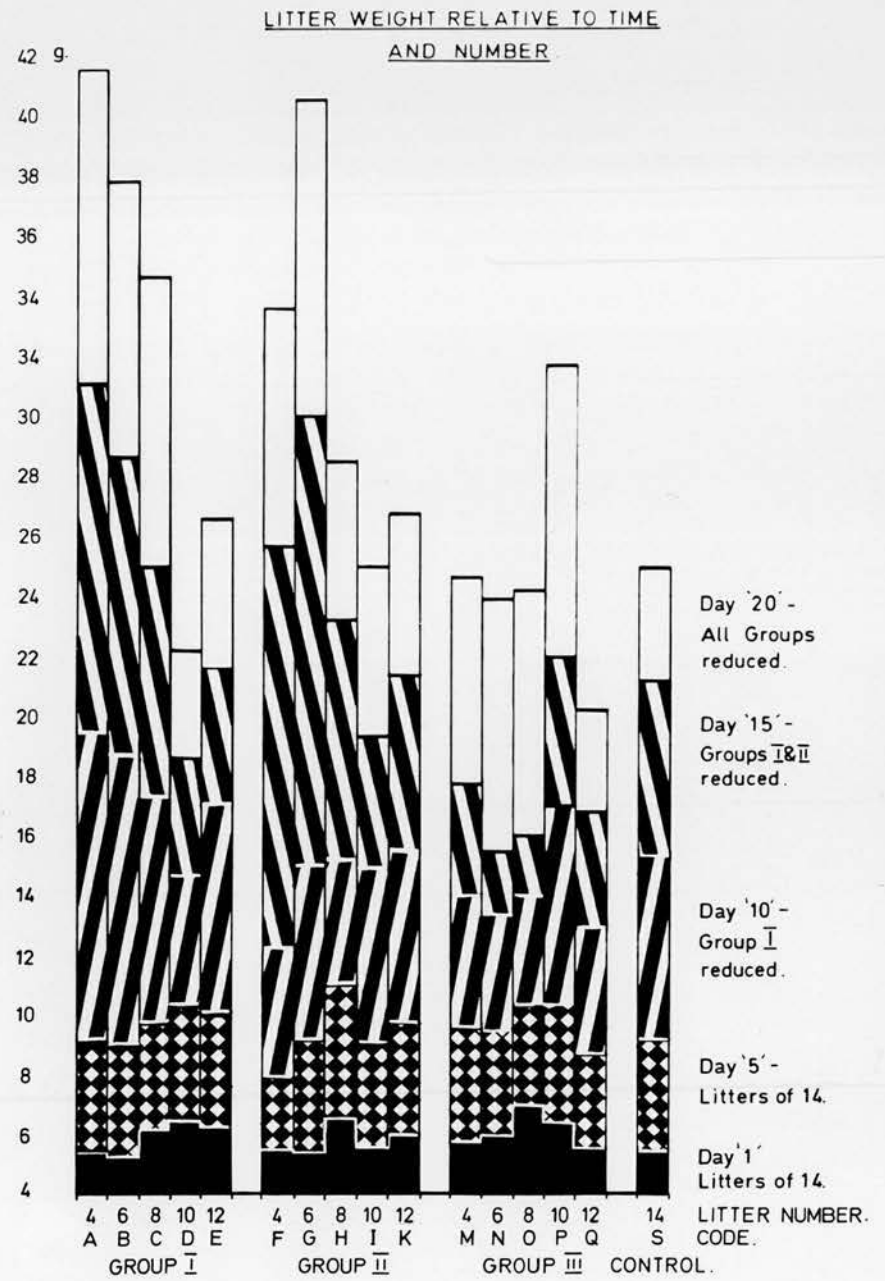

LITTER WEIGHT RELATIVE TO TIME
AND NUMBER
42 g.
40
38
36
34
34
30
28
26
24
22
20
18
16
14
12
10
8
6
4
Day '20' -
All Groups
reduced.
Day '15' -
Groups I&II
reduced.
Day '10' -
Group I
reduced.
Day '5' -
Litters of 14.
Day '1'
Litters of 14.
4 6 8 10 12 4 6 8 10 12 4 6 8 10 12 14 LITTER NUMBER.
A B C D E F G H I K M N O P Q S CODE.
GROUP I GROUP II GROUP III CONTROL.

TABLE 4

Code	Reduct. Day	Orig. No.	Reduced No.	Regres. Line 1 - 5 Days			Regres. Line 5 - 10 Days			Regres. Line 10 - 15 Days			Regres. Line 15 - 20 Days		
				X1	X5	b	X5	X10	b	X10	X15	b	X15	X20	b
A	5	14	4	5.42	9.06	0.901	8.69	18.97	2.057	18.97	29.96	2.198	31.44	42.04	2.191
B	5	14	6	5.21	8.93	0.927	8.45	18.23	1.954	18.81	28.44	1.927	29.12	38.24	1.823
C	5	14	8	6.18	9.66	0.871	10.31	17.52	1.441	17.35	25.05	1.540	24.69	34.18	1.897
D	5	14	10	6.51	10.28	0.944	10.32	15.07	0.948	15.08	18.80	0.744	18.70	22.16	0.691
E	5	14	12	6.32	10.03	0.933	10.37	17.43	1.413	16.83	21.59	0.953	21.69	26.42	0.946
F	10	14	4	5.30	7.72	0.604	8.38	12.73	0.871	13.33	26.49	2.633	26.40	32.98	1.318
G	10	14	6	5.27	9.07	0.950	9.25	15.21	1.911	15.18	29.99	2.963	29.97	40.26	2.058
H	10	14	8	6.31	10.96	1.161	11.13	15.56	0.886	15.75	23.71	1.591	22.69	31.63	1.075
I	10	14	10	5.60	9.08	0.871	8.84	14.37	1.105	14.26	19.07	0.964	19.25	24.62	1.072
J*	10	14	12	5.56	8.16	0.650	8.55	10.82	0.453	10.67	13.50	0.566	13.01	14.56	0.310
K	10	14	12R	5.97	9.56	0.899	10.02	15.10	1.016	15.64	21.55	1.182	21.47	26.44	0.994
L*	15	14	4	6.98	10.22	0.811	9.77	13.77	0.801	13.38	14.71	0.267	15.00	25.97	2.195
M	15	14	4R	5.77	9.50	0.934	9.70	14.19	0.897	14.40	17.92	0.703	16.72	24.22	1.501
N	15	14	6	6.05	9.64	0.899	9.99	13.09	0.776	13.69	15.52	0.377	14.71	23.48	1.756
O	15	14	8	6.77	10.10	0.833	10.44	14.03	0.717	14.05	16.20	0.429	16.24	23.61	1.473
P	15	14	10	6.38	10.22	0.961	10.18	16.96	1.356	17.36	22.12	0.951	22.22	31.59	1.873
Q	15	14	12	5.54	8.63	0.773	8.72	12.99	0.852	13.01	16.86	0.768	16.27	20.10	0.767
R*	15	14	12R	5.58	8.00	0.606	8.07	10.37	0.461	10.48	13.91	0.686	13.87	16.44	0.514
S	-	14	14	5.42	9.13	0.926	8.36	14.68	1.265	14.90	21.89	1.398	21.51	25.13	0.726

N.B. Line 1 - 5 14 in Litter in ALL, i.e. A to S
Line 5 - 10 14 in Litter F to S, others as stated in col. 4
Line 10 - 15 14 in Litter L to S, " " "
Line 15 - 20 as stated in column 4

*These data were not used since common regression lines between the replicates could not be constructed.

TABLE 4 (Cont'd)

Code	Reduct. Day.	Orig. No.	Reduced No.	Regres. Line 1 - 10 Days			Regres. Line 1 - 15 Days			Regres. Line 5 - 20 Days			Regres. Line 10 - 20 Days		
				X1	X10	b	X1	X15	b	X5	X20	b	X10	X20	b
A	5	14	4	-	-	-	-	-	-	8.00	41.96	2.264	-	-	-
B	5	14	6	-	-	-	-	-	-	8.53	38.53	2.000	-	-	-
C	5	14	8	-	-	-	-	-	-	9.93	33.21	1.552	-	-	-
D	5	14	10	-	-	-	-	-	-	10.91	22.47	0.771	-	-	-
E	5	14	12	-	-	-	-	-	-	11.33	26.71	1.025	-	-	-
F	10	14	4	5.18	12.43	0.806	-	-	-	-	-	-	14.91	34.53	1.962
G	10	14	6	5.27	14.75	1.053	-	-	-	-	-	-	16.21	41.28	2.507
H	10	14	8	6.86	15.58	0.969	-	-	-	-	-	-	16.53	28.47	1.194
I	10	14	10	5.60	13.84	0.916	-	-	-	-	-	-	14.06	24.48	1.045
J*	10	14	12	5.89	10.94	0.561	-	-	-	-	-	-	11.03	14.75	0.372
K	10	14	12R	5.94	14.94	1.001	-	-	-	-	-	-	15.88	26.66	1.072
L*	15	14	4	-	-	-	7.71	15.56	0.560	-	-	-	-	-	-
M	15	14	4R	-	-	-	6.02	18.39	0.884	-	-	-	-	-	-
N	15	14	6	-	-	-	6.69	16.37	0.692	-	-	-	-	-	-
O	15	14	8	-	-	-	7.33	16.94	0.686	-	-	-	-	-	-
P	15	14	10	-	-	-	5.98	22.56	1.184	-	-	-	-	-	-
Q	15	14	12	-	-	-	5.49	16.98	0.821	-	-	-	-	-	-
R*	15	14	12R	-	-	-	5.58	13.61	0.576	-	-	-	-	-	-
S	-	14	14	-	-	-	-	-	-	-	-	-	-	-	-

N.B. Line 1 - 5 14 in Litter in ALL, i.e. A to S
Line 5 - 10 14 in Litter F to S, others as stated in col. 4
Line 10 - 15 14 in Litter L to S, " " "
Line 15 - 20 as stated in column 4

*These data were not used since common regression lines between the replicates could not be constructed.

and P and Q were sufficiently different to contra-indicate the construction of common regression lines pair-wise. For further analytical purposes, the code litters K, M and Q were selected on the basis that their growth appeared to be more uniform due to the more stabilised quality of the maternal influence of the "mothers".

Group 1 (litters A to E) when examined on day 15 showed clearly that gain in weight was inversely proportional to the number contained in the litter. On the other hand, litters D - 10 and E - 12 appear to be "out of phase" with E gaining more weight than D, yet their respective weights on day 15 are of the same order as the weights in Group 3 (M to Q) and that of the control litter (S). On the evidence presented it appears that the reduction of a litter to 10 rats is not particularly rewarding.

By day 15 the litters of Group 2 (F to K), which were reduced some 5 days previously on day day (Figs. 15, 23) clearly show a rapid gain in weight for litters F - 4, G - 6 and a little less for litter H - 8. Once more it is noticeable that litters I - 10 and K - 12 show no distinctive advantages over litters containing 14 young. Within Group 2 it is evident that the "mother" of litter G - 6 is a particularly good one - if the evidence of the rapid gain in weight is of significance.

Litters of Group 3 (M to Q) show a considerable variability with the exception of litter P - 10 which at this particular stage shows a greater weight increase than that observed in the control litter S. As noted in the section dealing with Methodology, the data connected with control litter S has been derived from Experiment 1 where evidence showed that the "mother" was exceptionally good - thus setting a rather high standard.

Examination of the data on day 20 for Groups 1 (A to E) and 2 (F to K), confirms the observations and implications noted on day 15. Group 3 (M to Q),

which was reduced in numbers - M - 6, N - 6, O - 0, P - 10 and Q - 12 (Figs. 15, 18) shows conclusively that a reduction, even at this late stage of events, makes a considerable contribution to the overall weight gain achieved by smaller litters as compared to the control litter S. One litter within the group - Q - 12 - appeared to be the one exception and did not benefit by the changes, whereas litter P - 10 which had already given evidence of a good "mother" continued the trend with further increases.

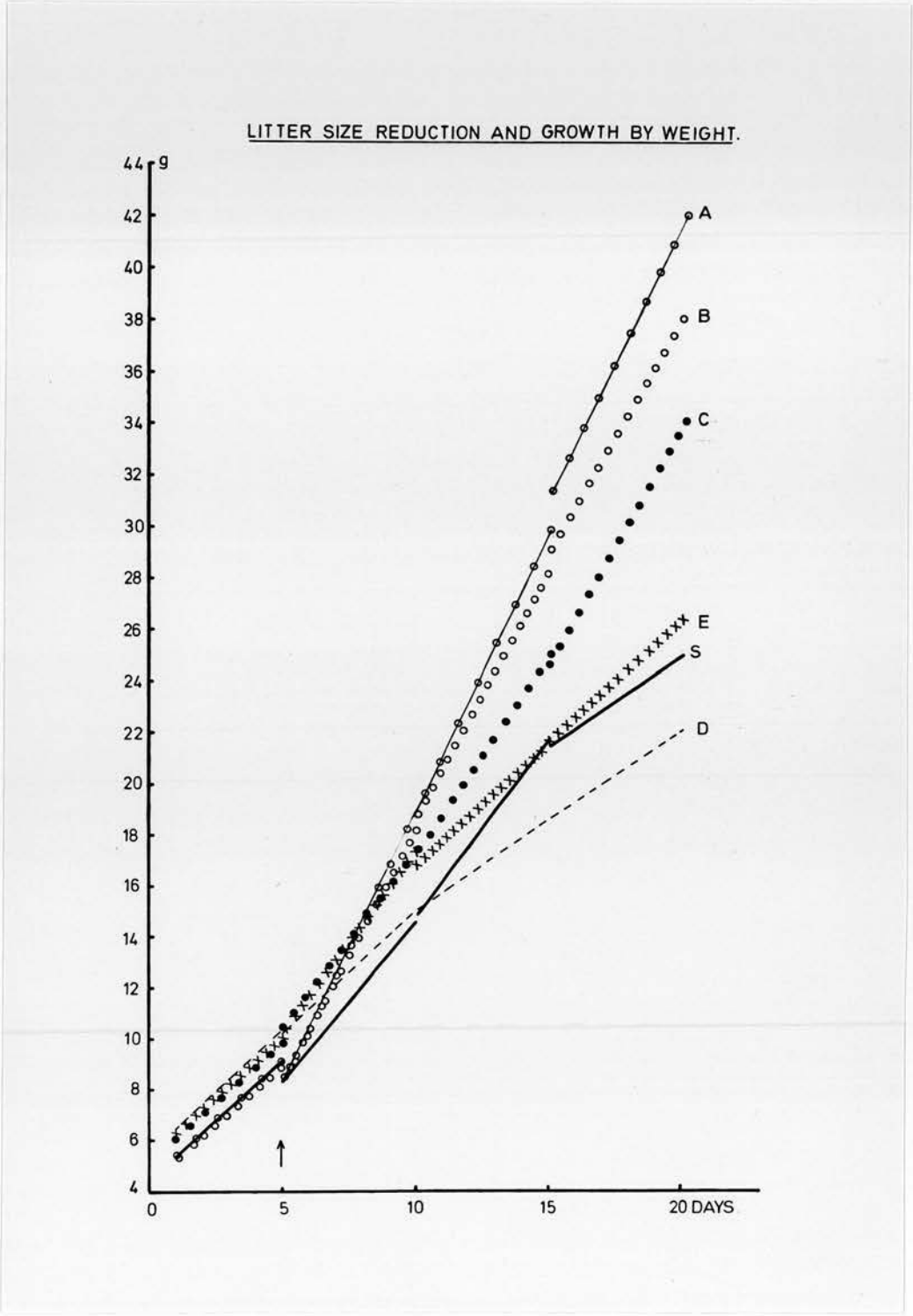
Growth rate is best expressed graphically in the form of linear regression lines, the slope of the lines being based on the coefficient of the regression "b", or as a more precise definition, "b" is the estimate of the slope of the rectilinear regression equation, the sample coefficient of the regression. Regression lines constructed for the litters in Group 1 (A to E) are shown in Figure 16.

Rates of growth over the period 1-5 days, when all these experimental litters consisted of 14 young, appear to be similar in that differences initially present continued to higher levels while maintaining their relative positions to one another. This point is demonstrated by the similarity of parallelism which many of the lines exhibit.

Examination of the period 5-10 days shows clearly that the most rapid gain in growth is achieved by the smallest litter A - 4, while the remaining litters of B - 6, a slightly slower growth, and C - 8 and E - 12, a slow growth, lag a little behind. Litter D - 10 has a growth rate which indicates that the lactational capacity and general maternal behaviour is of poorer quality hence the progress of the litter did not fit into an ordered rosette of 4, 6, 8, 10 and 12.

Within the periods of 10-15 days and 15-20 days, the growth rates emerging for the small litters of A - 4, B - 6 and C - 8 appear to be fairly uniform, however, the change of growth rate in D - 10 and E - 12 suggests that the amount

Figure 16 Growth rates of litters in Group 1 (A to E) together with the control litter (S) represented by linear regression lines based on the coefficient of the regression b. Growth examined over the periods of 1-5, 5-10, 10-15 and 15-20 days. All experimental litters started with 14 young at birth and were reduced on day 5 as follows: A to 4, B to 6, C to 8, D to 10 and D to 12 young per litter. Calculations based on mean weight.


LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.
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15
20 DAYS
A
B
C
E
S
D

of reduction which has occurred is of too low an order to induce much difference. Indeed, when comparisons are made with the control litter S - 14, the reduction on day 5 of the litters D - 10 and E - 12 seems to have little effect since their overall growth presents a similar spectrum.

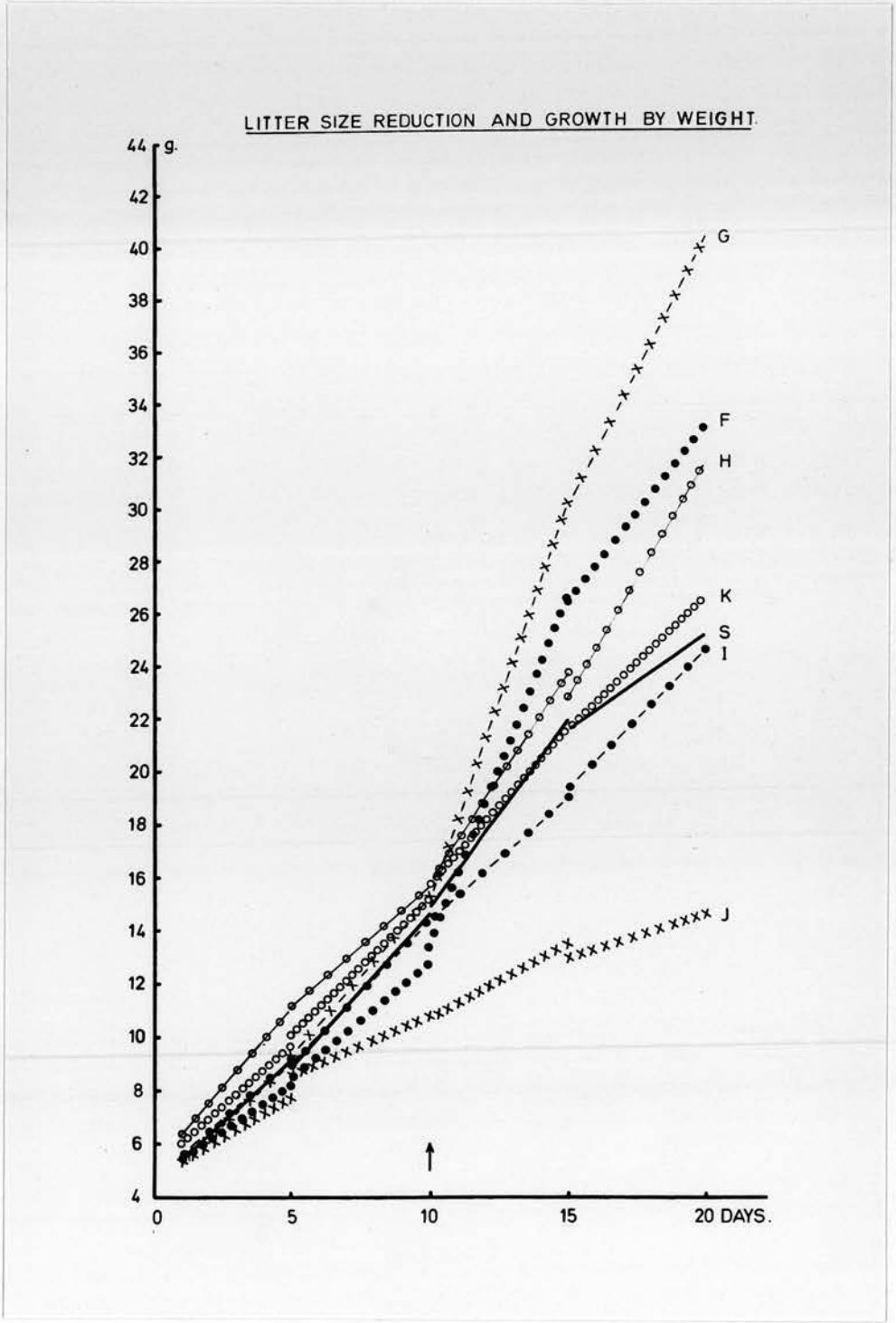
Group 2 (F to K) shows clearly from the constructed regression lines (Figs. 17, 24, 25) that litter J - 13 is unusually poor in its development. The growth rate, which is fairly constant up to day 10, i.e. the day of reduction, for all litters, shows very rapid increases for litters G - 6, F - 4 and H - 8, in that order, and indicates that litter G - 6 obviously has a good "mother". The remaining litters of I - 10 and K - 12 do not differ significantly from the growth observed in the control litter S - 14 and thus confirm the findings noted previously at the day 5 reduction of litters.

Finally, Group 3 (L to R) when assessed from their regression line construction (Figure 18) indicates that up to day 15, when all the litters were composed of 14 individuals, most of the experimental litters are inferior to the outstandingly good control litter S with perhaps the exception of litter P which appears to be marginally better. Litter R is, without doubt, very much inferior. Following the litter reduction on day 15, the growth rate based on the slope of the lines shows dramatic growth increases except for litters Q and R. Litter P - 10 also exhibits a similar increase. In the final analysis, however, this particular surge of growth is not sufficient in the time available to compensate for the previously experienced undernutrition stemming from the large numbers of individuals in litters. The final weights, therefore, are much smaller than in the previously examined two groups.

3.8 Experiment II. Maternal Capacity and Stimulation - Discussion

The growth of rodents during the suckling period is influenced by their own genes, as well as by various environmental effects, and a portion of these

Figure 17 Growth rates of litters in Group 2 (F to K) together with control litter (S) represented by linear regression lines calculated from the coefficient of the regression b based on mean weight. Growth examined over the period 1-20 days and all experimental consisted of 14 young from birth and were reduced on day 10 as follows: F to 4, G to 6, H to 8, I to 10 and J and K to 12. Note that G (with 6 young) has a good mother while J (with 12 young) makes little headway.


LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.
g.
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12
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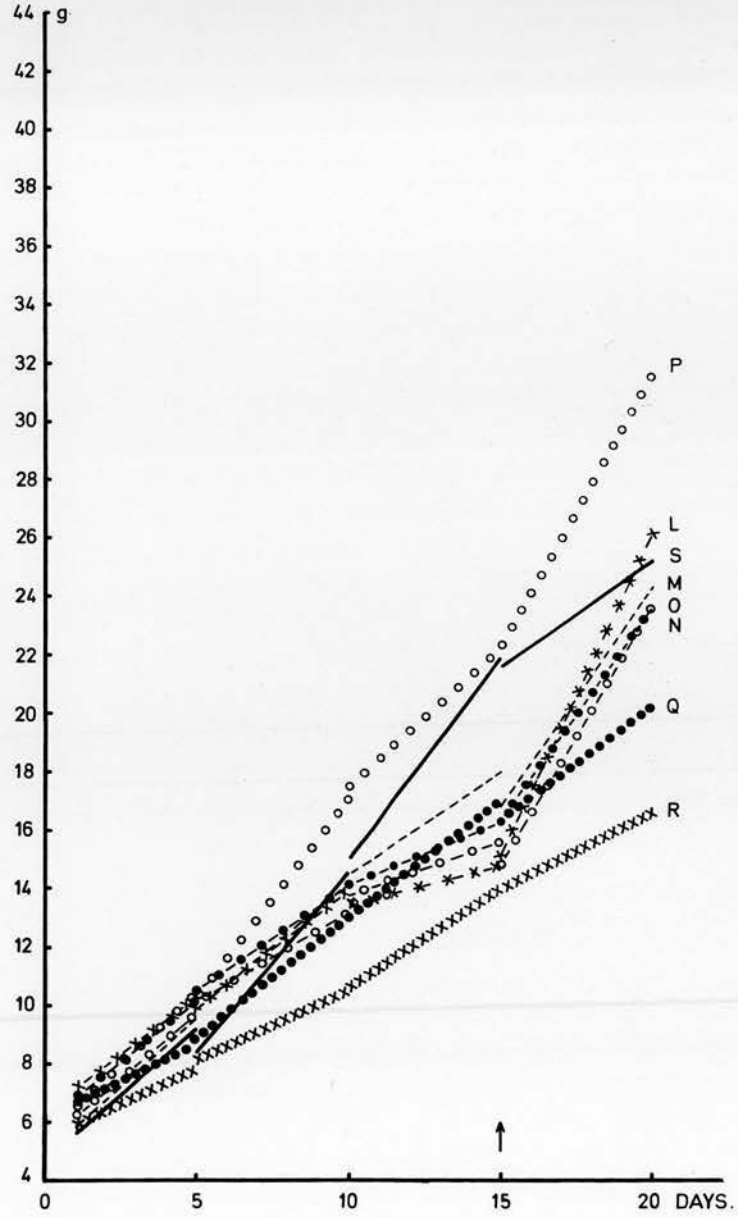
4
0
5
10
15
20 DAYS.
G
F
H
K
S
I
J

Figure 18 Growth rates of litters in Group 3 (L to R) with the control litter (S) represented by linear regression lines based on the coefficient of the regression b. Growth observed over the period 1 to 20 days. All experimental litters retained with 14 young until day 15 when they were reduced as follows: L and M to 4, N to 6, O to 8, P to 10, Q and R to 12. Following the litter reductions rapid growth occurs in all the litters except in Q and R.

LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.



may be attributable to the genotype of the mother. The influence of a mother upon her young during the prenatal period is fully expected, but what is not often realised is, however, the full extent that the maternal environment, i.e. nursing, lactational capacity, etc. influences the growth rate of the young during the preweaning stage.

The maternal environment can be divided into prenatal and postnatal factors - in this instance the postnatal stage remains within the province of the preweaning phase, i.e. the period during which active suckling occurs. As LEGATES (1972) points out, various factors such as temperature and maternal instincts are of importance although the main influence on growth is the lactational capacity.

Next of interest concerns the factors influencing the lactational capacity of the mother. The physiological aspects with their attendant hormonal background do not involve us in the present context, but those factors which can be tentatively expressed as "experience" and "stimulation" can be related directly to the size of the litter. Experience can be judged qualitatively and quantitatively after the female rat has given birth to several litters. This so-called experience will add greatly to any future lactational and maternal performance but will not replace the "milk-ejection" reflex (CROSS and HARRIS, 1952) based on nipple stimulation.

Varying effects of maternal behaviour have been studied by SEITZ (1954) and in his initial findings, based on litters of 6 and 12 young, the mothers given large litters behaved less maternally towards their "offspring" than mothers given small litters. Explanations for this phenomenon included the point that usually when reinforcement principles are operating - the greater the number of young, the greater the stimulation - but since the greater numbers induced a negatively reinforced maternal reaction in the mother, the question of maternal

fatigue arises. Thus we have an inverse correlation between litter-size and maternal behaviour with the underlying lactational influence.

Further work by SEITZ (1958) was designed to investigate whether the inverse relationship between litter-size and maternal behaviour could be found for mothers with successive litters of different sizes. The findings revealed that with each increase in litter-size there was a corresponding decrease in maternal behaviour. The main statistically significant differences appeared between litter-sizes of 3 and 9, and between 6 and 12. From these results the deduction emerged that the relationship between litter-size and maternal behaviour occurred in steps and could be regarded as almost a linear inverse correlation.

Growth rate in rats has been shown to be inversely proportional to litter-size (PARKES, 1929; KENNEDY, 1957; WIDDOWSON and KENNEDY, 1962; PARK, 1969; PARK and NOWOSIELSKI-SLEPOWRON, 1971, 1972; NOWOSIELSKI and PARK, 1973, 1974). When the difference between the litter-size is extensive, body weight, body and skull lengths and the development of the vibrissae, showed marked retardation in the large litters at weaning (KENNEDY, 1957a, b; WIDDOWSON and McCANCE, 1960; DOBBING, 1964; PARK, 1969, 1970; PARK and NOWOSIELSKI-SLEPOWRON, 1971, 1972; NOWOSIELSKI-SLEPOWRON and PARK, 1973).

Originally (PARK, 1968) while investigating aspects of oral development, the size of litters caused problems in the variable growth rates noted in the individual young so that the litter-size was maintained at 6 or 7 rats per litter. In more recent papers (PARK, 1969; PARK and NOWOSIELSKI-SLEPOWRON, 1971, 1972; NOWOSIELSKI-SLEPOWRON and PARK, 1973) concerned with growth during the pre- and postweaning phases of development, a small litter of 6 rats was used and was regarded as representing the optimum incremental conditions. These litters were reduced or formed of 6 individuals at birth or day 1. More recent work

of Experiment 1 indicates that the pre-selected number of individuals per litter of 6 may indeed be the correct number, however, it also emerged that the total litter weight of litters of 7 or more young appear to remain fairly constant as if the maximum milk yield had been achieved. The number of individual progeny, however large, have to share this amount of milk with the result that weight will fall with the increase of numbers. Also noticeable from Experiment 1 is the inability of small litters consisting of 2 or 3 individuals to stimulate the optimal lactational rate - this being made evident by their total and average weights being considerably lower than those achieved by larger litters (Fig. 26).

Growth of young rats during the preweaning phase of development can depend not only on the maternal environment, but on the size of the litter in that environment. Generally, it is accepted that within a small litter each rat should be able to obtain enough milk to allow its development to move at its maximum potential, whereas if the litter is large with competition occurring between its members for a fixed milk supply, then the natural sequence is a lack of milk in varying degrees per rat and hence a similar varying lack of growth. Milk supplies, however, is not maintained at any one level since it has been found (REDDY and DONKER, 1964; KUMARESAN, ANDERSON and TURNER, 1967) that as the litter size increases, so also does the milk production but not in a direct relationship to the larger numbers in the litters.

A young rat has a genetically inherent capacity for growth which cannot be exceeded unless we move into the realms of deposition of adipose tissue. Under this influence, the presence of more milk than required should not basically alter the growth of the rat. With the differing sizes of litters there must exist a range of young rats which will all grow at their maximum for a normal mother, and that this particular number in the litter would be forced to be less with a mother of lower maternal capacity or endowment.

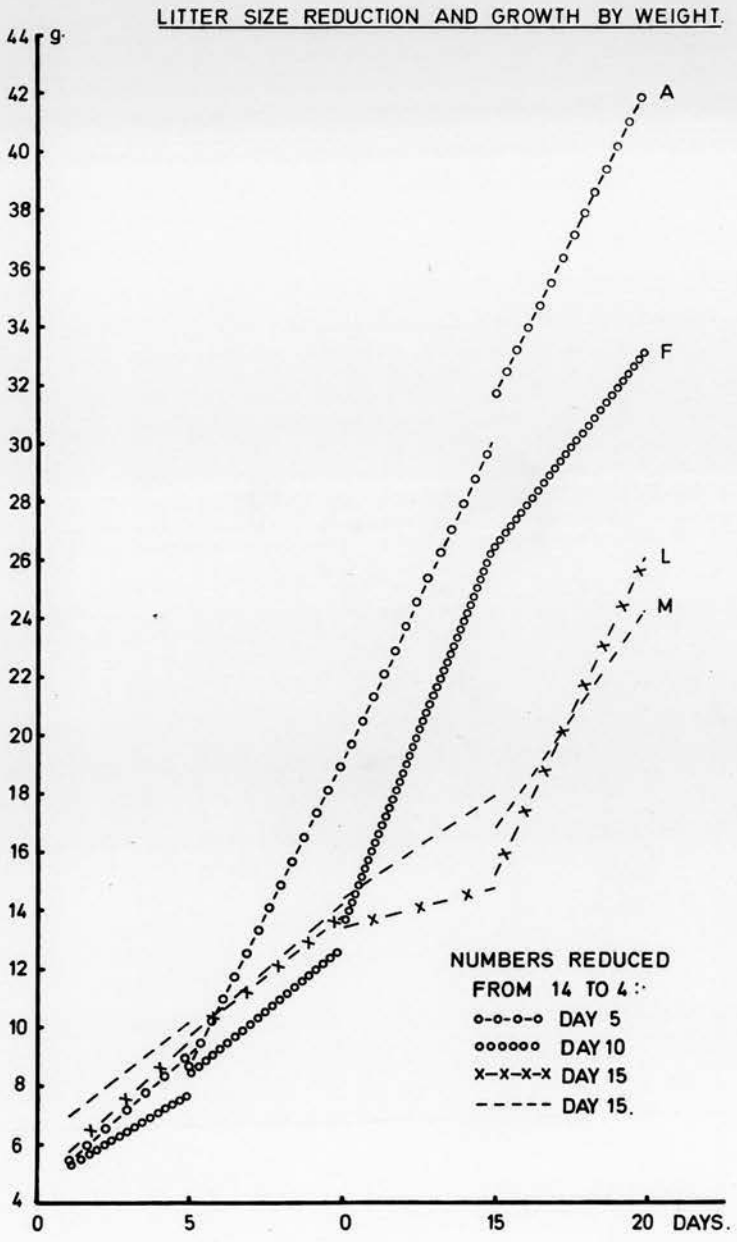
Examination of the results clearly indicates that the reduction of the size of the litters must make available an ample supply of milk which is expressed by the rapid gain of weight of these litters. Reductions of the litters of 14 down to small litters of 4, 6 or even 8 on day 5 or day 10 of the preweaning period appear to be the most beneficial in regard to growth. Smaller reductions of 14 down to 12 or 10 individuals per litter - irrespective of the time of reduction - produce no significant returns.

Analysis of those litters reduced from 14 young to 4 on selected days of 5, 10 and 15 is demonstrated by the regression line construction in Figure 19. There can be little doubt in the final assessment that reduction on day 5 is optimal, however, it should be noted that the slope of the regression line following the day of reduction is always greatest indicating a phase of rapid weight gain.

Litters reduced from 14 young to 6 on days 5, 10 and 15 are analysed by linear regression line construction in Figure 20. From inspection it is obvious that litter G - which was reduced on day 10 - achieves the greatest weight increase together with that achieved by B - reduced on day 5 - which follows closely. Litter N - reduced on day 15 - shows a reverse of this trend by being nearly 18g. lighter.

Figure 21 demonstrates the regression analysis of litters reduced from 14 individuals to 8 on day 5, 10 and 15 and which can be regarded as the remaining litter-changes based on Table 4 and Figures 16, 17, 18 and 19 which hold interest of a significant nature. The weight increase of those reduced on day 5 are greater than those reduced on day 10, although once the incremental difference had emerged it remained fairly constant with the final weights within 2.5 g. In contrast litter O reduced on day 15 lies some 8 g below that of litter N which was reduced on day 10.

Figure 19 Growth rates of litters reduced from 14 to 4 on selected days of 5, 10 and 15 (A on day 5, F on day 10 and L and M on day 15). The regression line analysis clearly indicates that a reduction on day 5 stimulates the greatest growth.


LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.
g.
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18
16
14
12
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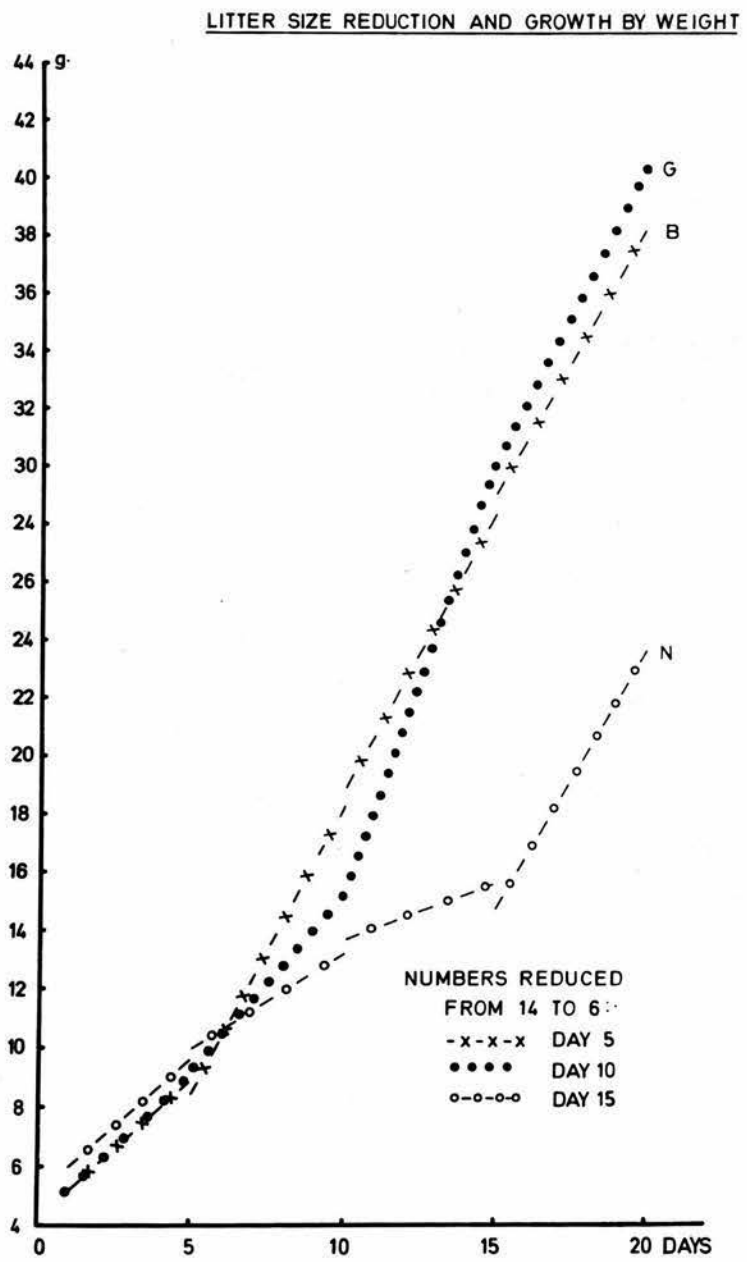
8
6
4
A
F
L
M
NUMBERS REDUCED
FROM 14 TO 4 :-
o-o-o-o DAY 5
oooooo DAY 10
x-x-x-x DAY 15
- - - - - DAY 15.
0
5
0
15
20 DAYS.

Figure 20 Growth rates of litters reduced from 14 to 6 young on selected days of 5, 10 and 15 (B on day 5, G on day 10 and N on day 15). Although litter G was reduced 5 days after B, it is obvious that it grows much faster. This difference may lie in maternal variance.


LITTER SIZE REDUCTION AND GROWTH BY WEIGHT
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G
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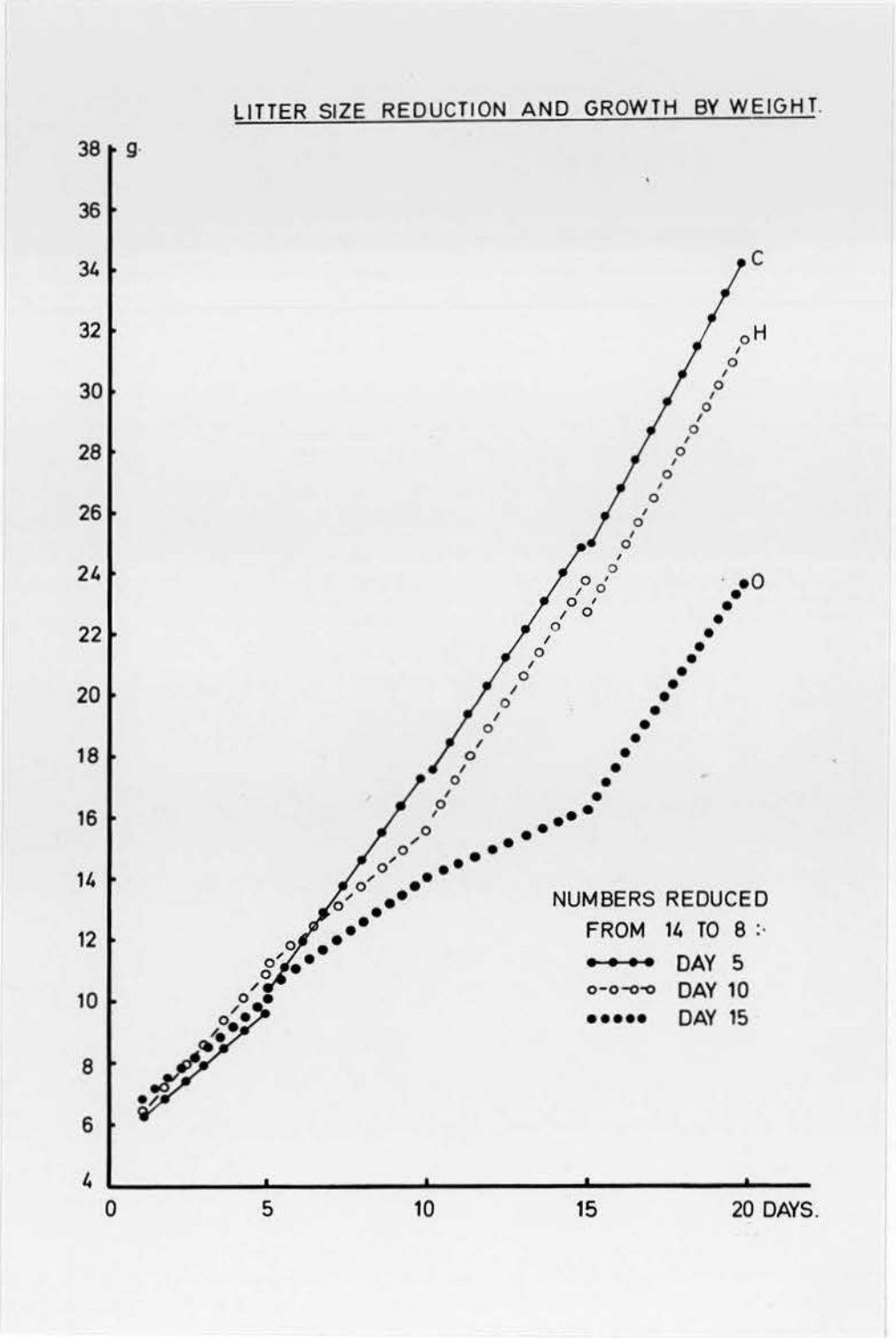
NUMBERS REDUCED
FROM 14 TO 6
-x-x-x DAY 5
●●●● DAY 10
o-o-o-o DAY 15
0
5
10
15
20 DAYS

Figure 21 Growth rates of litters reduced from 14 to 8 young on the selected days of 5, 10 and 15 (C on day 5, H on day 10 and O on day 15). Regression analysis shows that C has a greater growth rate and is followed by H. In contrast, O although showing some rapid growth after reduction remains inferior.


LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.
g.
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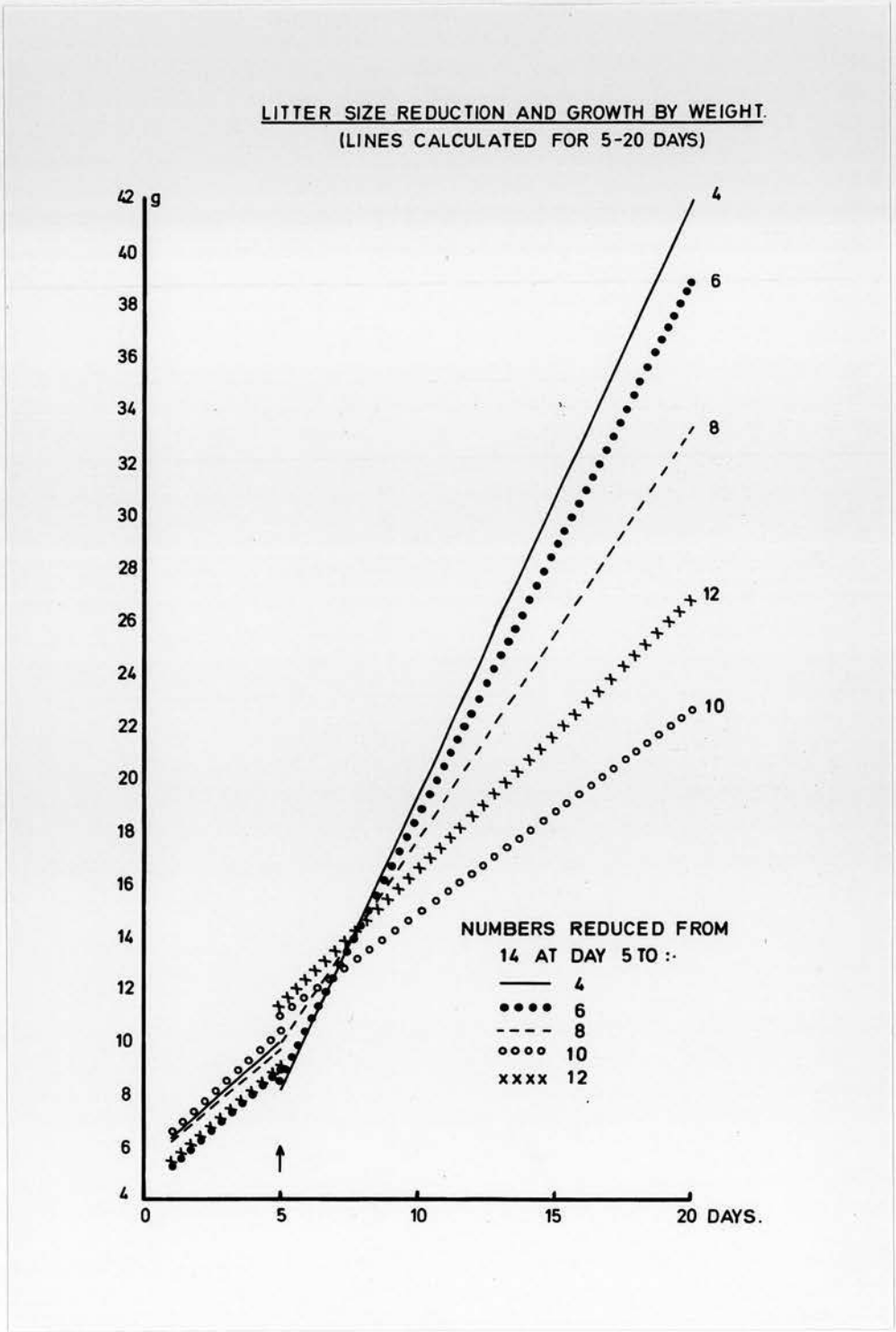
15
20 DAYS.
C
H
O
NUMBERS REDUCED
FROM 14 TO 8 :-
DAY 5
DAY 10
DAY 15

Figure 22 Growth rates of litters in Group 1 (A to E) represented by regression lines calculated for 5-20 days. All the litters were maintained at 14 young and then reduced on day 5 as follows: A to 4, B to 6, C to 8, D to 10 and E to 12. The smaller litters thus formed - 4, 6 and 8, all showed a rapid increase of growth by day 10 compared to the remaining larger litters.


LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.
(LINES CALCULATED FOR 5-20 DAYS)
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NUMBERS REDUCED FROM
14 AT DAY 5 TO :-
4
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8
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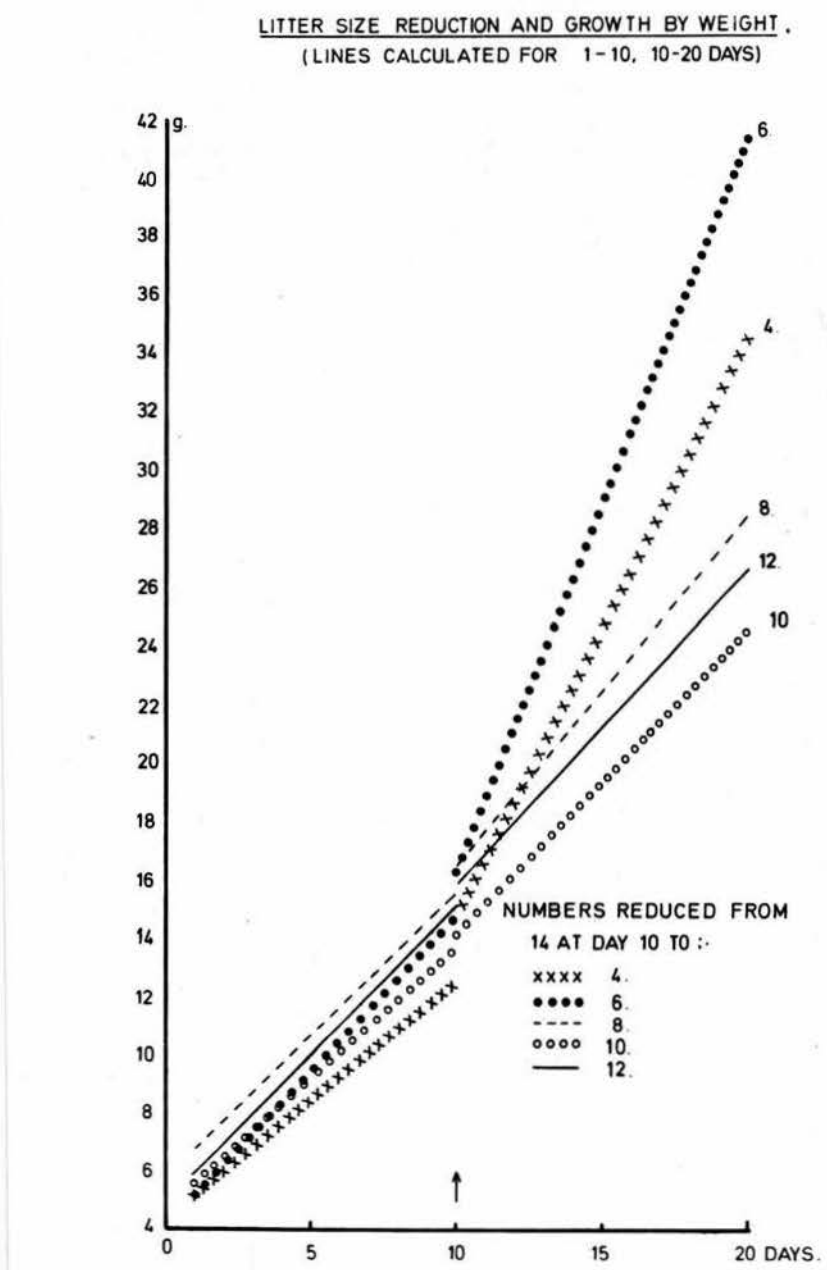
12

Figure 23 Growth rates of litters in Group 2 (F to K) represented by regression lines calculated for 1-10, and 10-20 days. The litters were maintained at 14 young and then reduced on day 10 as follows: F to 4, G to 6, H to 8, I to 10, K to 12. The maternal capacity of the mother controlling the litter of 6 (G) is obviously high. For comparisons with the control litter S see Figures 15 and 17.


LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.
(LINES CALCULATED FOR 1-10, 10-20 DAYS)
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NUMBERS REDUCED FROM
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oooo 10.
—— 12.

Figure 24 Growth rates of litters in Groups 1, 2 and 3 represented by regression lines. The litters were maintained at 14 young and then reduced to 12 on days 5, 10 and 15 as follows: Day 5, E to 12; Day 10, J and K to 12; Day 15, Q and R to 12. The earlier the reduction as noted in E (Day 5) has obvious benefits. The fact that K, which was reduced on Day 10 has a similar final attainment reflects the maternal capacity of that particular mother.

LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.

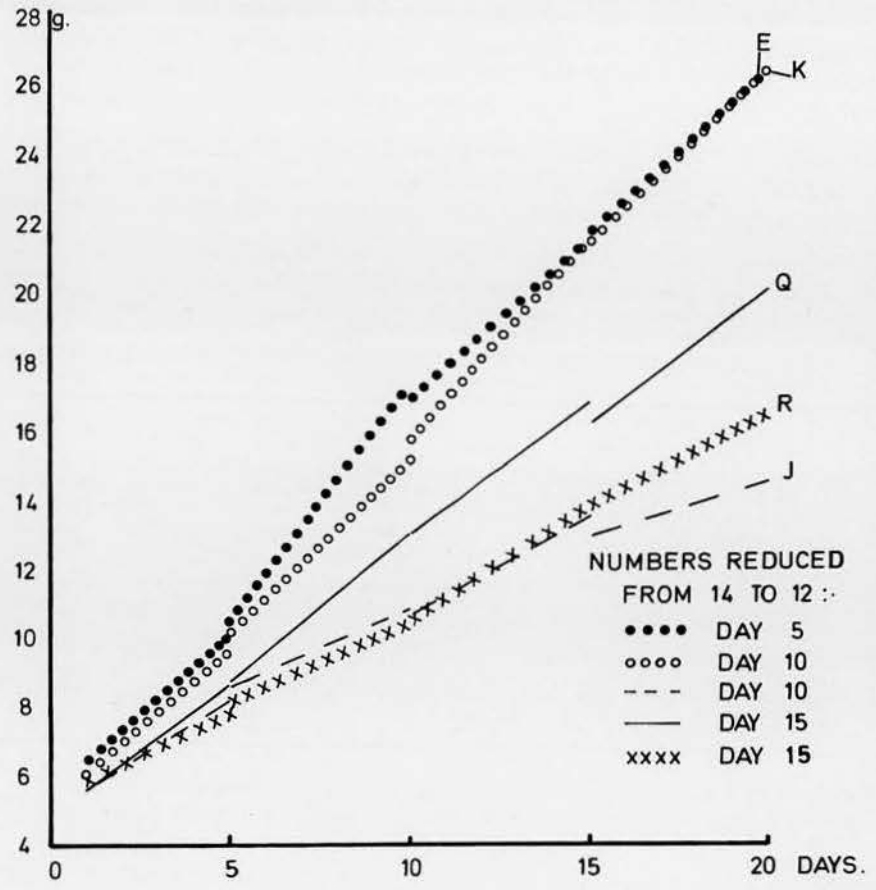


Figure 25 Growth rates of litters reduced from 14 to 10 young on selected days of 5, 10 and 15 represented by regression line analysis based on weight. Code D on day 5, I on day 10, and P on day 15. The growth of D indicates a poor lactational capacity while I appears to have made little gain when compared with the control in Figure 15. In P there is firm evidence that the "mother" has a good maternal capacity.

LITTER SIZE REDUCTION AND GROWTH BY WEIGHT.

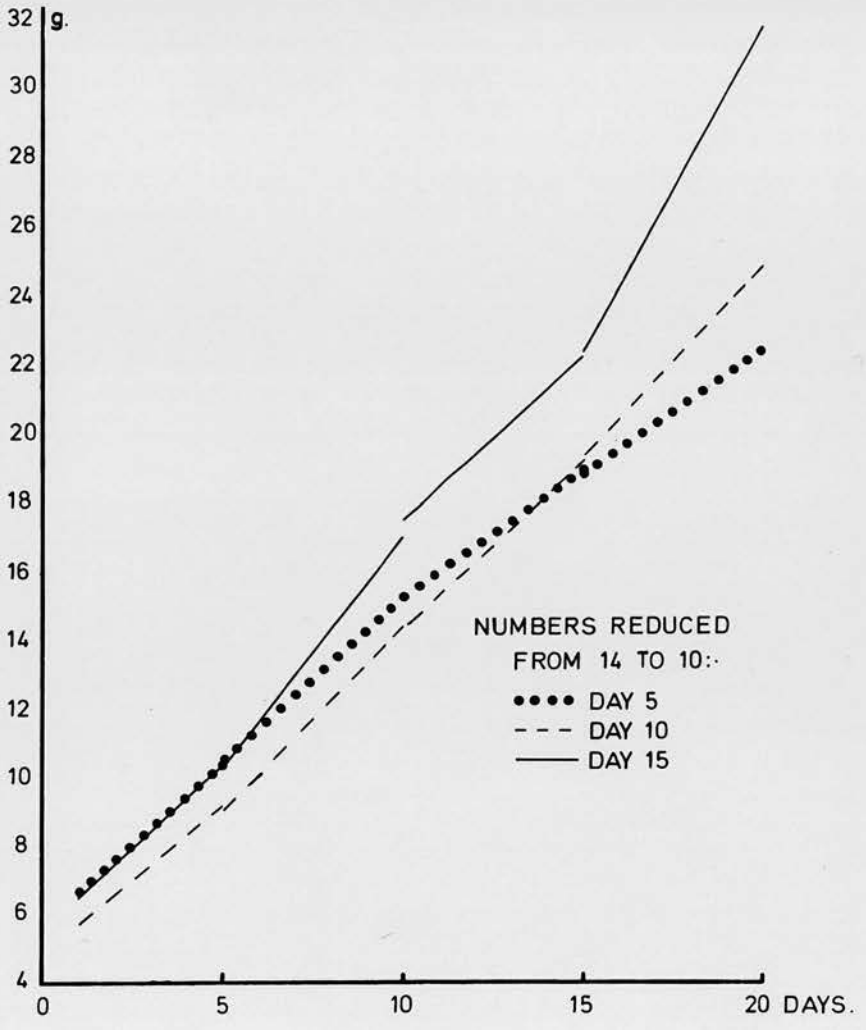
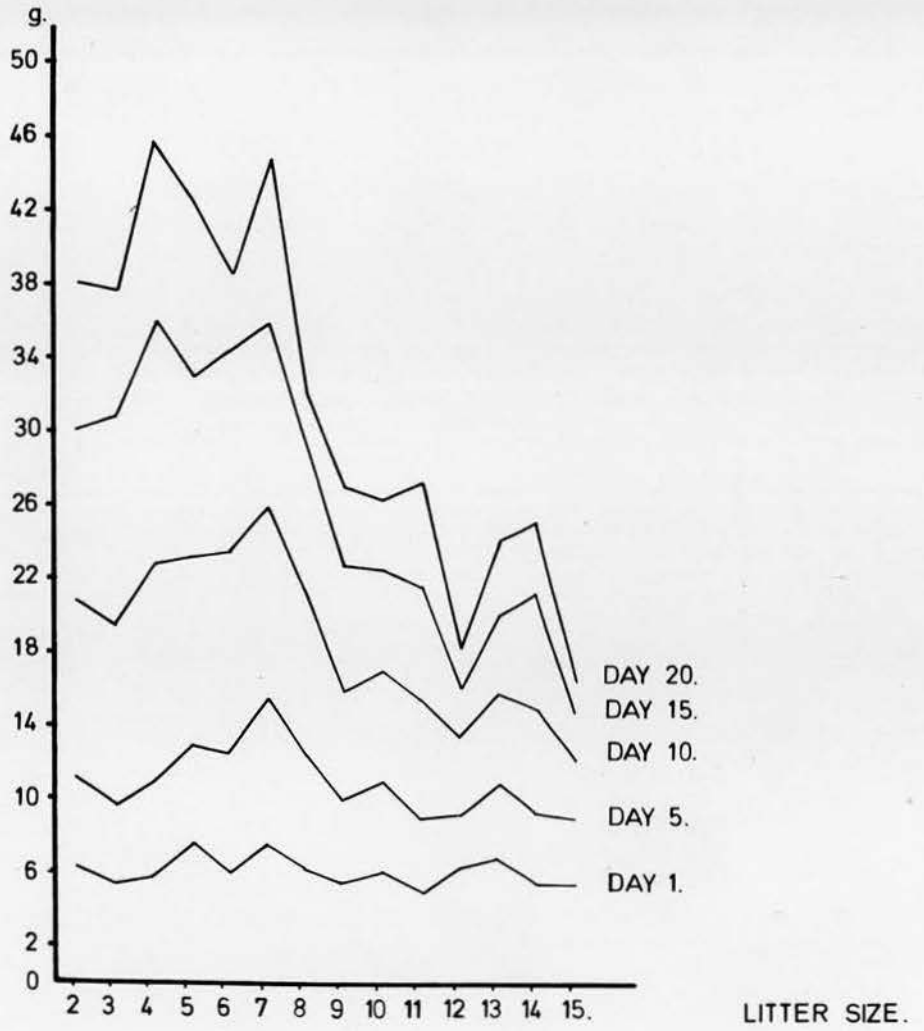


Figure 26 A synopsis of the average weight of young rats in litters of various sizes and the levels reached on days 1, 5, 10, 15 and 20. Calculations were based on the data used in Experiment 1 (Section 3.3. to 3.5) and clearly shows that the large litters reduced the amount of milk available, the average sized litters thrived while the small litters of 2 or 3 appeared to be unable to stimulate lactation.

AVERAGE WEIGHT OF INDIVIDUAL IN LITTERS OF VARIOUS SIZES.



The method of rearing small litters containing 1 to 8 young should, theoretically, allow them to obtain sufficient milk to attain the maximum potential growth for any reasonable mother. There remains the problem, however, of the lower numbers if the mother has not received sufficient stimulation. This particular point was noted by BRODY (1942) where a litter containing 1 young rat often resulted in a failure of the mother to lactate.

Some indications relating to factors influencing lactation have emerged from the work of EISEN and HANRAHAN (1970) who investigated the genotypic and dietary (high energy versus standard diets) effects on the lactational performance of both mother and litter weights of mice. The high energy diet led to significant increases in litter weight and milk yield which, on the surface, suggests that the "energy" was being conveyed by the milk. However, one of the more interesting findings was that the percentage of solids and lipid in the milk did not differ significantly between control and experimental mothers but rather that the high energy diet led to a greater milk quantity.

An investigation into the body weight and food consumption of rats nursing various sizes of litters was conducted by OTA and YOKOYAMA (1967) and it was found that irrespective of the sizes of the litters the rate of increase of body weight of the adult rats was similar. The food intake of the mother rats appeared to be related to the weight gain of the young rats and had a linear relationship, and further to this it was noted that the food intake of the mothers increased as well as the gain in litter weight when the number of young rats per litter increased. A postulate was made that the increase in the weight of the mother rat was not related to the food intake or the litter-size but to changes of the ovarian function during lactation.

Composition of rat milk has been studied by MUELLER and COX (1946) in relation to changes in diet and it was found that a decrease in protein caused a

reduction in the volume and fat content of the milk but that the protein concentration did not alter - milk always containing 9 to 10% protein. When adult lactating rats were fed on very high fat or grain diets, the milk content contained a percentage of fat of approximately 30% and 13% respectively (SCHEMMEL, MICKELSON and FISHER, 1973). Within the present experiment, the dietary regime of the adult rats remain constant in terms of quality while the quantity available remained within the province of the individual rat's choice or preference.

Following parturition the amount of milk secreted begins to rise and it is thought to reach a maximum at about 12 to 14 days - although some observations suggest that it could be near the 10 day mark - after which a gradual decline sets in. MOON (1962) regarded three factors within a single nursing or milking period as the controlling influences. These factors involved: (1) the number of alveolar cells per gland, (2) the functional activity of each cell, and (3) the degree of milk letdown activity.

It is now generally accepted that the stimulus of suckling releases both galactopoietic and galactokinetic hormones from the pituitary gland (MOON, 1962, 1969; EDWARDSON and EARYS, 1967; TUCKER, PAAPE and SINHA, 1967). Mammary cell proliferation can be measured by the content of DNA (deoxyribonucleic acid) (MOON, 1962) which was found to increase relative to increases of milk and suckling stimulation. Work by GROSVENOR, KRULICH and McCANN (1968) and MENA and GROSVENOR (1968) showed that increasing amounts of pituitary somatrotrophin and prolactin appeared when the number of suckling young were raised. Thus the number of suckling young has a regulating effect on the amount of milk produced, for example, the larger litters had a greater weight gain (REDDY and DONKER, 1964, OTA and YOKOYAMA, 1967) and the increased DNA content in the mammary gland stemmed from increased litter-size (TUCKER, 1964, 1966; MOON, 1965). Evidence of the

effects of litter-size relative to their gain in weight ranges from the failure of a single rat to stimulate lactation (BRODY, 1942) to the medium sized litters reaching their maximum growth potential, and the larger litters showing growth retardation due to inadequate milk supplies. Although stimulation by increasing the litter-size does have an effect on the availability of milk, a limit is reached whereby the number of young and the milk production no longer have a direct relationship (REDDY and DONKER, 1964; KUMARESAN, ANDERSON and TURNER, 1967).

The essential variable, so far not considered, is that of the mechanism of milk delivery. The basic gland tissue and its DNA content remains similar when the stimulation of a rat nursing 12 young is maintained, compared to that of a rat nursing 6 young (MOON, 1965). On further analysis with different litter sizes (MOON, 1969) it was found that the DNA content of the glands increased with larger litters and that the milk yield was also greater. Thus it was postulated that the greater the suckling stimulus (large litters) the greater the DNA (gland cellular content) and hence the greater the milk production. However, the problem remains that the large litter, in spite of the relative increase of milk supplies, does not show a relative gain in weight. From this it can be inferred that there must be a maximum "compensatory" increase of milk due to greater stimulation and that beyond this level it is not possible to increase the production.

Two explanations have been postulated by KUMARESAN, ANDERSON and TURNER (1967) and these involve the maternal behaviour and the glandular contraction mechanism. In the glandular mechanism the controlling factor is generally regarded as oxytocin which is released from the posterior pituitary gland in response to neural stimulation via the suckling process (ELY and PETERSEN, 1941; WHITTLESTONE, 1950, 1952; WHITTLESTONE, BASSETT and TURNER, 1952a, b; GROSVENOR and TURNER, 1956, 1957, 1959a, b; MORAG, 1968, 1970; MORAG and

BRICK, 1969). The action of the oxytocin on reaching the gland is to cause the contraction of the myoepithelial tissues (SWANSON and TURNER, 1941; RICHARDSON, 1949; LINZELL, 1952) around the alveoli and small ducts so that the milk is pushed into larger ducts. An essential point is that this suggestion was extended by a further postulate that oxytocin was also linked with the bioxynthesis of milk (BENSON and FOLLEY, 1956). The glactopoetic role of oxytocin has been further studied by a number of workers (KUMARESAN and TURNER, 1966; MEITES, 1958; McCANN, MACK and GALE, 1959; MEITES and NICOLL, 1959; MEITES and HOPKINS, 1961; MORAG and BRICK, 1969; MORAG, 1970). There is, therefore, the possibility that even with sufficient stimulation, the level of oxytocin released is not enough to act upon the glands and thus prevent milk secretion so that a large litter, having raised the overall milk production by stimulation, is prevented from using their maximum growth potential.

The second explanation is linked to the behavioural pattern of nursing and to the young rat's inherent consumption. Maternal behaviour for nursing young consists of a feeding session of 1 to 5 minutes at approximately hourly intervals and allowing that the glands are full, a large litter could quite easily empty them within the time - this does not mean that all young rats will receive equal amounts. If there is a lack of sufficient oxytocin or even an absence then the glands could not be emptied in the time available. There are three other variables which should be considered: (a) the feasibility of all the mammary glands functioning at maximum capacity, (b) the young rats within the litter having differing "personalities", i.e. some having a greater dominant drive combined to physical strength thus making milk distribution uneven since the stronger and more motivated rat is able to feed more quickly and for longer. This point is supported by the range of different weights of rats within one large litter, (c) in addition to the limited nursing period regulated by the

release of oxytocin, it is possible that the voluntary consumption of milk by the young rat varies just as in growing and mature rats, thus a large supply of milk will not increase their intake or influence their growth rate (GROSSIE and TURNER, 1961).

Assessment of litter growth by gain in weight is, at best, an indicator of the trend for the initial appearances may be one of constance yet consist of different rates. Weight expresses growth in 3-dimensions but, unlike length, is much more sensitive to change and hence more variable. The search for a standardised litter exhibiting similarity in growth rate depends on the age of the animal used. If a number of litters are required for study ranging in age from birth to 20 days, then the litter-size should fall within the region of 5, 6 or 7 animals. To maintain litter-sizes above or below these particular numbers introduces considerable growth fluctuations. For investigations of pre-weaning rats which can be left for a few days prior to active experimentation, the standardisation can be improved by forming initially large litters thus raising the lactation stimulation factor, and then reducing the number of rats down to the 5, 6 or 7 level. By alteration of the litter-size after initial stimulation, one is ensuring an adequate milk supply suitable in quantity and quality to encourage the rats to reach their individual maximum growth potential. The use of a large litter to encourage a rise in lactation must be tempered with prudence since if the litter-size is too large then the individual members may have degrees of retardation before the litter-size is reduced. The basic requirement is to achieve sufficient stimulation of the mother which will result in a greater availability of milk without causing disturbances to the overall growth rate before reducing the size of the litter. The selection of such a litter might be based on the presence of 12 teats, but there are indications that this number of rats only thrives if the lactational capacity of

the mother is outstanding. As a tentative figure, the litter-size should never be more than 8 individuals which should be within the capabilities of even a moderate mother.

3.9 Experiment II. Maternal Capacity and Stimulation - Summary

In Experiment 1 (Sections 3.2 to 3.5) the search for variables controlling the development of young rats dealt with the influence of the numbers in the litters and the relation of them to the maternal environment. Emerging from this was the establishment of the "standardised" litter of 5-6 young per mother. It is known that there is an inverse correlation between litter-size and maternal behaviour with the underlying lactational influence. There are also suggestions that the natural reduction of the litter-size by infant mortality enables the remaining young to utilise both the extra milk available and their own growth potential.

An experiment was evolved to examine the maternal capacity in relation to the stimulation of the mother by using large litters followed by reducing the litters at selected periods and noting the growth pattern of the smaller litters so formed. The experimental litters stemmed from 18 female rats selected on their proven breeding and maternal capacity characteristics. The young rats at birth were mixed and reallocated to their "mothers" (cross-fostering technique, (KENNEDY, 1957a, b; WIDDOWSON and McCANCE, 1960)) to form litters consisting of 14. Changes to this size of litter took place on days 5, 10 and 15 - based on the observations of the triphasic growth pattern (PARK, 1972; PARK and NOWOSIELSKI-SLEPOWRON, 1972) - and were initiated so that litters consisted of 4, 6, 8, 10 and 12 young.

Thus on day 5: Litters coded with letters A to E were reduced as follows:-
A to 4, B to 6, C to 8, D to 10 and E to 12 rats per mother.

Day 10: Litters coded with letters F to K were reduced to:

F to 4, G to 6, H to 8, I to 10, J and K to 12.

Day 15: Litters coded L to R were reduced to:

L and M to 4, N to 6, O to 8, P to 10, Q and R to 12.

Following their formation each litter was weighed at 24-hourly intervals. The control litter used for comparison was annexed from Experiment 1 and designated with an S.

When the mother was stimulated by a large litter, the lactation increased relative to the size of the litter. Following a short period of stimulation the increased milk supply could be used to benefit the litter, but only if the litter was reduced in numbers. The litter-size making the best use of the milk was found to range between 5 and 7 young. Litters above this number did not show a very uniform progress. The timing of the litter reduction was crucial and had to be made on day 5, thus a "standardised" growing rat with ample milk supply for full use of its growth potential is only possible after five days. The size of the stimulatory litter of 14 also appeared to be crucial since this number resulted in some instances in a growth retardation occurring before any reduction in the number had been possible. From the results it is possible to postulate that a stimulatory litter should consist of 8 rats at birth and that to achieve the fullest growth potential the subsequent litter-size should consist of 5 or 6 individual rats per mother.

Details of the physiological and maternal behavioural patterns in relation to lactation and litter-size were discussed in the light of current hypotheses and awareness made of some of the complications arising out of the interactions of these two main controlling factors.

PART IV

PATTERNS OF BODY GROWTH IN RELATION TO LITTER SIZE

As a natural phenomenon is only the expression of ratios and relations and connections, at least two bodies are necessary to its appearance. So we must always consider, first, a body which reacts or which manifests the phenomenon; second, another body which acts and plays the part of environment in relation to the first. It is impossible to imagine a body wholly isolated in nature; it would no longer be real, because there would be no relation to manifest its existence.

CLAUDE BERNARD

4.1 Patterns of Body Growth - Introduction

The principle of equifinality has an important application in the growth of the body as a whole when related to the effects of maternal environment and extremes of litter-size. In animal growth it is generally accepted that following a temporary cessation of growth and from varying initial sizes, that the ultimate species-characteristic size can still be attained. The one proviso to this is that damage to the growing tissue does not reach the level of preventing normal function and growth when normal conditions return.

An example of a temporary suspension of growth will be observed when diet is lacking in both quantity and quality and has been investigated experimentally using rodents and other animals. It was found that growth either stopped or slowed down so that there was no evidence increases in body weight although it appeared to be maintained at a reasonable level. When normal diet was resumed the animals eventually reached the normal expected final weight (KOPEC, 1938; CLARKE and SMITH, 1938; JACKSON, 1939; BERTALANFFY, 1960). With litters of varying size the weight of the animal at birth is normally low when it is a member of a large litter, whereas in a small litter the weight is greater. A slight clarification is needed at this juncture because there are lighter individual animals such as rats at birth when the litter is large sometimes due to the length of birth - the first born are sometimes feeding before the last born have emerged. In the circumstances the separation of lighter or heavier litter members is perhaps better viewed following 1 day postnatal since it is the availability of milk supplies which enable growth to continue. There are many factors which could influence the size of an animal at birth so that this kind of generalisation must be accepted, however, it has been shown (KOPEC, 1932) that the same final weight is eventually attained by both large and small animals at birth.

The growth of the young during the preweaning period (birth to 20 days) can be retarded by the nutritional influence of inherited differences in milk production by the mother so that prior to weaning any differences in the size noted at birth may be either accentuated or maintained, or even created over that period. The observations of HAZEL, BAKER and REINMILLER (1943) and KNAPP and CLARKE (1947, 1950) show that the retarded growth trend in various animals eventually became reversed and a normal final weight was reached over a period of time. This type of recovery of growth only occurs if the period of malnutrition has not inflicted lasting damage on the developing tissues.

It is known that severe undernutrition in early life often results in permanent stunting of growth in children and experimental animals (McCANCE and WIDDOWSON, 1962; WIDDOWSON and McCANCE, 1963; CRAVIOTO, 1963; DOWNS, 1964; PARK, 1968). The extent to which the animal recovers depends upon the age of onset and the duration of the nutritional deficits (SCHULTZE, 1955; WIDDOWSON and McCANCE, 1963; WINICK and NOBLE, 1966; BROWN and GUTHRIE, 1968). An example of this was noted in natural large litters (PARK, 1968) where a number of rats, originally retarded during the preweaning period, were found to be unable to regain their deficits in weight and length during the postweaning period when food was more than adequate. This type of backward development is regarded by WIDDOWSON and KENNEDY (1962) as being related to the increased hereditary control over gain in weight and size after weaning and to the rate of multiplication which decreased with time.

Originally, the effect of litter-size on rat body growth was studied (PARK and NOWOSIELSKI-SLEPOWRON, 1971) and the two factors of genetic endowment and maternal environment suggested as the controlling influences. The variation in the maternal environment was eliminated as far as possible by the reduction of the litter-size thus ensuring that an adequate milk supply would be available, and by mating 2-3 times the females which had by their behaviour with their

first litters justified the title of "proven" mothers. Variation in growth was found to be minimal hence the postulate that the influence of the maternal environment was a predominant factor. When the converse was attempted using large and therefore under-fed litters, a much wider variation in growth was found which could be attributed to the unequal division of the inadequate milk supply. Further, it was noted that while adequate feeding produced rats of relatively uniform size, inadequate feeding gave rise to well-marked individual differences. The appearance of this variation was at first attributed to their individual genetic function but this explanation is not sufficient since an unequal division of milk supplies leads to similar results.

Preweaning growth is complex, being influenced by the genotype of the young, as well as by the postnatal maternal environment - mainly attributable to the phenotype and experience of the mother. Using mice, FALCONER (1947) indicated that the 12-day weight of a standardised litter might give a measurement of lactation. Work bearing on this suggestion has shown that the 12-day litter weight can be used as a phenotypic measure of the postnatal maternal influence on the growth of the young since approximately 60% of the variation in individual 12-day weight is due to the effect of the postnatal mother (COX, LEGATES and COCKERHAM, 1959; YOUNG, LEGATES and FARTHING, 1965; EL OKSH, SUTHERLAND and WILLIAMS, 1967). Recently, however, EISEN, LEGATES and ROBINSON (1970), and EISEN and HANRAHAN (1970), have indicated that much of the maternal variation for the individual 12-day body weight, normally regarded as the province of the mother's phenotype, is caused by either environmental or non-additive genetic effects. These workers examined the genotypic and dietary (high energy versus standard) influences on lactational performance on both mother and litter weights of mice selected for increased 12-day litter weight. Results showed that a high energy diet led to heavier litters, but since there was no change in the percentage of

solids or percentage lipid content in the milk, the rise in the weight of litters fed by mothers on a high energy diet stemmed from a greater milk yield.

The preweaning phase of development of the rat is generally regarded as lasting at least 20 days although the dictum appears to rest on when one separates the mother from the young. Biological weaning must be a transitional phenomenon which begins as the maximum milk supply starts to decline and the intake of food from other sources replaces the increasing deficits. From a general view the maternal environment has a considerable influence on the growth occurring within the 20 day span. In a previous sample, the effects of litter-size on body growth (PARK and NOWOSIELSKI-SLEPOWRON, 1971) were investigated and the means of the daily weights were plotted on a semi-log scale so that the difference between the rates of growth between the small well-fed litters and the large under-fed litters could be clearly demonstrated. These plots showed that they could be divided into three discrete lines with different slopes indicating changes in the growth rates. Reasons for the appearance of three phases of growth during the first 20 days could be attributed to maternal environment (milk) - maternal environment and food from other sources - food from other sources only and a rising genetic endowment. The aim of the present chapter is to confirm or refute the preweaning body growth spectrum.

4.2 Patterns of Body Growth - Litter Selection

The samples examined were derived from two separate breedings of albino laboratory rats (Sprague-Dawley strain) - the first occurring in 1968-69 (PARK, 1969; PARK and NOWOSIELSKI-SLEPOWRON, 1971) consisting of 192 (small litter) rats and 333 (large litter) rats ranging from birth to 20 days - and designated as Sample 1. The second breeding took place during the period 1971-72 and consisted of 246 (small litter) rats and 317 (large litter) rats over the same range and being designated as Sample 2. In addition, small and large litters were continued for a further 20 days, i.e. 20 to 40 days to allow insight into the

possible "recovery" rate of the rats belonging to larger litters. This particular aspect of older rats will be dealt with in a separate section of this chapter.

As stated in Materials and Methods (Section 2.4), the animals were obtained from a 24-hourly series of rats formed on the basis of large and small litters. Breeding was arranged to ensure that the greatest number of litters possible would be born within the same period of time. The essential factors were to use "proven" mothers and to obtain one or more litters within the same 6-8 hours of birth or less if possible. These litters were then mixed together to distribute as evenly as possible any potential genetic differences in growth before selection and formulation into their experimental litters. Although differences in sex did not affect the growth trend, the small litters were formed of 6 rats with equal numbers of the sexes. The large litters, designed to highlight the effects of under-nutrition on growth, were formed in a similar manner into litters ranging from 13-17 with equality of numbers of the sexes where possible. The large litters (based on the work of KENNEDY, 1957a, b, and WIDDOWSON and McCANCE, 1960) by their very size obtained a gross reduction of the milk normally available by overtaxing the maternal capacity.

4.3 Patterns of Body Growth - Preweaning Observations

Data stemming from the measurements of length and weight of the small and large litters arising from both samples of rats showed, following construction of regression lines based on the daily arithmetic means, that they differed considerably from curves fitted by eye. What was evident was that the weight and length grew faster in the small litters than in the large litters. It was found that it was not possible to construct meaningful curvilinear regression lines to the same formula as lines fitted by eye were of various shapes, broadly based on log growth curves. Not only were these curves of various shapes although the fitting was applied to the same parameter, e.g. length which showed

differences in shape between small and large litters, but also between the small litters of both samples. Thus it was clear that it was not possible to use the same formula for the same parameter. Another point of interest was that the data obtained from the large litters was more heterogenous than that of the small litters. As a further check, the difference data, i.e. the means of the large litter measurements subtracted from the means of the small litters, also indicated that these relations were not linear in type. After a close examination of the curves fitted by eye superimposed on linear regression lines of the data of (a) small litters, (b) large litters, and (c) the differences, suggested that linear regression lines could be constructed but that the line should consist of three separate parts, thus three linear regression lines could be used to express a set of records for a discrete period, e.g. day 1-5, day 5-15 and day 15-20.

The breakdown of the total regression line was suggested either by means of the largest overall difference of the regression coefficient "b" or by selecting the mid-day in the curve where "b's" had a small difference on consecutive days with an abrupt drop on either side of such a sequence. The results calculated for length are given in Table 5 while those for weight are given in Table 6, covering both samples.

Tables 7 and 8 give the analysis of the individual litters covering the small well-nourished litters (6 individuals - 3 males and 3 females with a number of replicate litters), and large under-fed litters (ranging from 13-18 individuals with sexes arranged in approximately equal numbers) based on weight in grams. Tables 9 and 10 summarise the results stemming from the head-body length measurements of both small and large litters. Further tables (Tables 11 and 12) are presented to demonstrate the daily gain in weight and head-body length of the small and large litters together with the obesity and ponderal indices.

TABLE 5

LINEAR REGRESSION ANALYSIS - LENGTH

Regression Line	Small Litters		Large Litters	
	Sample 1	Sample 2	Sample 1	Sample 2
Day	1 - 20	1 - 20	1 - 20	1 - 20
Mean	8.37	8.88	7.46	8.12
Regression coefficient	0.3018	0.3344	0.2101	0.2439
Day	1 - 5	1 - 5	1 - 5	1 - 5
Mean	6.11	6.26	5.99	6.11
Regression coefficient	0.2710	0.4770	0.2720	0.4850
Day	5 - 17	5 - 17	5 - 14	5 - 15
Mean	8.56	9.11	7.14	8.12
Regression coefficient	0.3305	0.2993	0.1612	0.1969
Day	17 - 20	17 - 20	14 - 20	15 - 20
Mean	10.65	11.39	8.75	9.70
Regression coefficient	0.0670	0.4820	0.1484	0.2926

TABLE 6

LINEAR REGRESSION ANALYSIS - WEIGHT

Regression Line	Small Litters		Large Litters	
	Sample 1	Sample 2	Sample 1	Sample 2
Day	1 - 20	1 - 20	1 - 20	1 - 20
Mean	24.44	26.19	14.71	17.56
Regression coefficient	2.1771	2.4327	1.0376	1.3075
Day	1 - 5	1 - 5	1 - 5	1 - 5
Mean	8.91	8.95	7.27	7.56
Regression coefficient	0.9800	1.9040	0.7020	1.4670
Day	5 - 17	5 - 15	5 - 14	5 - 14
Mean	25.15	24.27	13.27	16.72
Regression coefficient	2.5110	2.5157	1.0190	1.3684
Day	17 - 20	15 - 20	14 - 20	14 - 20
Mean	42.06	40.07	21.06	26.10
Regression coefficient	1.5400	1.8923	1.7093	0.9554

TABLE 7

FIRST SAMPLE

WEIGHT ANALYSIS (Grms.)

Age in Days	SMALL LITTER Number	Mean	Range	S.E. of Mean	Coeff of Var. %	LARGE LITTER Number	Mean	Range	S.E. of Mean	Coeff of Var. %
0	6	6.03	5.7 - 6.2	0.0794	3.1	16	5.81	5.2 - 6.4	0.0241	5.2
1	6	6.98	6.7 - 7.3	0.0878	3.1	16	5.91	3.9 - 7.0	0.1671	11.3
2a	6	8.15	7.5 - 8.6	0.1723	5.2	15	6.09	5.1 - 8.2	0.2177	13.8
2b	6	7.53	6.8 - 8.1	0.2184	7.1					
2a + b	6 + 6	7.84	6.8 - 8.6	0.1622	4.0					
3a	6	9.00	8.3 - 9.5	0.1863	5.1	17	7.87	5.9 - 8.8	0.2297	12.0
3b	6	8.87	8.4 - 9.4	0.1540	4.2					
3a + b	6 + 6	8.93	8.3 - 9.5	0.1171	4.5					
4a	6	9.83	9.6 - 10.1	0.0854	2.1	18	8.03	6.2 - 9.7	0.2431	12.5
4b	6	10.20	9.7 - 11.2	0.2381	5.7					
4a + b	6 + 6	10.02	9.6 - 11.2	0.1206	4.2					
5a	6	10.57	9.9 - 11.0	0.1507	3.5	17	8.45	6.4 - 10.2	0.2402	3.7
5b	6	11.02	10.2 - 11.6	0.2025	4.5					
5a + b	6 + 6	10.79	9.9 - 11.6	0.1543	5.0					
6a	6	12.37	11.2 - 14.0	0.5857	11.6	15	10.70	9.9 - 12.1	0.2084	7.5
6b	6	13.70	11.9 - 15.4	0.5597	10.0					
6a + b	6 + 6	13.03	11.2 - 15.4	0.4354	11.6					
7	6	13.73	12.9 - 14.3	0.2122	3.8	18	10.08	8.9 - 12.2	0.2232	9.4
8	6	17.20	16.3 - 18.5	0.3099	4.4	15	11.37	9.5 - 13.2	0.2665	9.1
9	6	20.75	20.1 - 21.9	0.2859	3.4	15	10.75	9.0 - 13.6	0.3435	12.4
10a	6	20.88	20.3 - 21.6	0.2090	2.5	19	14.65	9.2 - 18.2	0.6088	18.1
10b	6	22.20	14.6 - 27.7	2.3337	25.7					
10a + b	6 + 6	21.54	14.6 - 27.7	1.1345	18.2					

TABLE 7 (Cont'd)

WEIGHT ANALYSIS (Grms.)

Age in Days	SMALL LITTER Number	Mean	Range	S.E. of Mean	Coeff of Var. %	LARGE LITTER Number	Mean	Range	S.E. of Mean	Coeff of Var. %
11	6	23.92	22.7 - 24.8	0.3082	3.2	16	15.19	13.0 - 16.4	0.2642	7.0
12	6	30.45	28.5 - 32.3	0.7565	6.1	15	16.94	12.1 - 19.1	0.4431	10.1
13	6	30.92	30.2 - 32.2	0.3071	2.4	17	20.81	17.4 - 27.2	0.5724	11.3
14a	6	31.38	29.7 - 32.1	0.3665	2.6	15	13.75	10.9 - 15.9	0.3402	9.6
14b	6	33.10	30.6 - 35.0	0.6871	5.1					
14a + b	6 + 6	32.24	29.7 - 35.0	0.4525	4.9					
15a	6	34.52	32.7 - 36.5	0.5997	4.3	18	16.51	13.4 - 19.7	0.3975	10.2
16a	6	37.63	36.9 - 38.3	0.2510	1.6	13	22.86	19.8 - 26.2	0.6243	9.9
17a	6	41.00	38.2 - 43.0	0.7362	4.4	15	22.21	19.7 - 26.2	0.4954	8.6
17b	6	39.33	37.1 - 41.1	0.5941	3.7					
17a + b	6 + 6	40.17	37.1 - 43.0	0.5852	5.1					
18a	6	40.15	39.3 - 41.3	0.3629	2.2	16	23.85	18.5 - 27.1	0.5489	9.2
18b	6	40.38	35.5 - 44.9	0.3622	8.3					
18a + b	6 + 6	40.27	35.5 - 44.9	0.6731	5.8					
19a	6	43.07	38.6 - 45.2	0.9931	5.7	13	23.49	21.6 - 25.0	0.2757	4.2
19b	6	44.07	39.0 - 47.9	1.2478	6.9					
19a + b	6 + 6	43.58	38.6 - 47.9	0.7313	5.8					
20a	6	47.43	46.8 - 48.2	0.2339	1.2	14	24.72	20.9 - 28.7	0.5656	8.6
20b	6	40.97	23.6 - 48.9	3.7367	22.3					
20a + b	6 + 6	44.20	23.6 - 48.9	2.0338	15.9					

TABLE 8

SECOND SAMPLE — WEIGHT ANALYSIS (Grms.)

Age in Days	SMALL LITTER Number	Mean	Range	S.E. of Mean	Coeff of Var. %	LARGE LITTER Number	Mean	Range	S.E. of Mean	Coeff of Var. %
0	6	6.03	5.7 - 6.2	0.0794	3.1	16	5.81	5.2 - 6.4	0.0241	5.2
1a	6	5.88	5.4 - 6.8	0.2287	9.53	17	5.21	4.6 - 5.6	0.0686	5.4
1b	6	5.73	5.4 - 6.1	0.1145	4.9					
1a + b	6 + 6	5.81	5.4 - 6.8	0.1241	7.4					
2a	6	6.42	5.6 - 7.1	0.2182	8.3	16	5.98	5.2 - 6.6	0.1127	7.5
2b	6	7.13	6.5 - 7.6	0.1543	5.3					
2a + b	6 + 6	6.78	5.6 - 7.6	0.1670	8.5					
3a	6	6.57	5.9 - 7.3	0.2335	8.7	17	6.80	6.4 - 7.6	0.0889	5.4
3b	6	8.88	7.7 - 10.5	0.4506	12.4					
3a + b	6 + 6	7.73	5.9 - 10.5	0.4249	19.0					
4a	6	12.52	10.8 - 13.7	0.3945	7.7	15	8.55	5.7 - 9.6	0.3066	13.9
4b	6	10.32	9.3 - 11.4	0.3782	9.0					
4a + b	6 + 6	11.42	9.3 - 13.7	0.4218	12.8					
5a	6	12.92	11.5 - 16.0	0.6651	12.6	15	11.26	6.7 - 13.1	0.4254	14.6
5b	6	13.10	12.3 - 13.8	0.2161	4.0					
5a + b	6 + 6	13.01	11.5 - 16.0	0.3345	8.9					
6a	6	11.87	10.5 - 13.0	0.3354	5.9	15	11.91	9.6 - 13.8	0.2696	8.8
6b	6	17.60	17.1 - 17.8	0.1034	1.4					
6a + b	6 + 6	14.73	10.5 - 17.8	0.8804	20.7					
7a	6	19.55	18.7 - 20.4	0.2706	3.4	15	13.84	10.4 - 16.4	0.4474	12.5
7b	6	18.72	18.1 - 19.6	0.2373	3.1					
7a + b	6 + 6	19.13	18.1 - 20.4	0.2126	3.9					

TABLE 8 (Cont'd)

WEIGHT ANALYSIS (Grms.)

Age in Days	SMALL LITTER Number	Mean	Range	S.E. of Mean	Coeff of Var. %	LARGE LITTER Number	Mean	Range	S.E. of Mean	Coeff of Var. %
8a	6	16.40	15.5 - 17.8	0.3425	5.1	15	14.29	7.1 - 17.7	0.6948	18.8
8b	6	17.00	15.6 - 19.3	0.5086	7.3					
8a + b	6 + 6	16.70	15.5 - 19.3	0.3059	6.4					
9a	6	20.25	18.4 - 22.7	0.6339	7.7	14	15.11	12.1 - 18.7	0.4478	11.1
9b	6	19.53	15.3 - 22.7	1.2550	15.7					
9a + b	6 + 6	19.89	15.3 - 22.7	0.6790	11.8					
10a	6	21.70	20.9 - 22.7	0.2978	3.4	13	15.83	13.5 - 18.4	0.4224	9.6
10b	6	24.85	23.4 - 26.3	0.5284	5.2					
10a + b	6 + 6	23.28	20.9 - 26.3	0.5560	8.3					
11a	6	24.85	23.4 - 25.4	0.2987	2.9	16	18.15	14.8 - 20.8	0.4409	9.7
11b	6	25.97	24.5 - 26.6	0.3063	2.9					
11a + b	6 + 6	25.41	23.4 - 26.6	0.2644	3.6					
12a	6	27.45	26.0 - 29.3	0.5488	4.9	13	20.88	18.0 - 22.3	0.3551	6.1
12b	6	28.38	27.2 - 29.4	0.3799	3.3					
12a + b	6 + 6	27.92	26.0 - 29.4	0.3479	4.3					
13a	6	32.57	31.7 - 34.3	0.3896	2.9	16	20.66	17.6 - 25.5	0.5388	10.4
13b	6	32.20	30.4 - 34.1	0.6708	5.1					
13a + b	6 + 6	32.38	30.4 - 34.3	0.3738	4.0					
14a	6	37.52	36.4 - 39.5	0.4331	2.8	13	25.22	20.1 - 27.6	0.5835	8.3
14b	6	31.68	30.7 - 34.3	0.5730	4.4					
14a + b	6 + 6	34.60	30.7 - 39.5	0.9437	9.5					
15a	6	39.35	36.5 - 42.8	0.8559	5.3	18	19.72	15.0 - 23.5	0.5237	11.3
15b	6	40.50	34.3 - 44.0	1.5889	9.6					
15a + b	6 + 6	39.93	34.3 - 44.0	0.8777	7.6					

TABLE 8 (Cont'd)

WEIGHT ANALYSIS (Grms.)

Age in Days	SMALL LITTERS Number	Mean	Range	S.E. of Mean	Coeff of Var. %	LARGE LITTERS Number	Mean	Range	S.E. of Mean	Coeff of Var. %
16a	6	36.72	32.2 - 41.0	1.3514	9.0	15	27.13	22.0 - 30.0	0.5517	7.8
16b	6	40.67	36.7 - 43.0	1.0197	6.1					
16a + b	6 + 6	38.69	32.2 - 43.0	1.0030	9.0					
17a	6	46.25	39.3 - 49.0	1.4426	7.6	15	27.80	24.0 - 31.4	0.6329	8.8
17b	6	46.95	43.4 - 49.8	0.8902	4.6					
17a + b	6 + 6	46.60	39.3 - 49.8	0.8150	6.1					
18a	6	39.83	36.8 - 42.1	0.7999	4.9	15	26.85	21.7 - 30.2	0.5297	7.6
18b	6	47.73	45.7 - 50.5	0.7424	3.8					
18a + b	6 + 6	43.78	36.8 - 50.5	1.2997	10.3					
19a	6	47.38	43.0 - 50.5	1.3035	6.7	14	25.90	21.3 - 31.1	0.7566	10.9
19b	6	41.17	36.3 - 45.2	1.2922	7.7					
19a + b	6 + 6	44.28	36.3 - 50.5	1.2822	10.0					
20a	6	57.05	55.6 - 59.0	0.5203	2.3	14	30.11	27.5 - 33.3	0.6269	7.8
20b	6	49.90	44.7 - 49.3	0.6817	3.4					
20a + b	6 + 6	51.98	44.7 - 59.0	1.5841	10.6					

TABLE 9

FIRST SAMPLE

LENGTH ANALYSIS (Cms.)

Age in Days	SMALL LITTERS					LARGE LITTERS				
	Number	Mean	Range	S.E. of Mean	Coeff of Var. %	Number	Mean	Range	S.E. of Mean	Coeff of Var. %
0	6	5.02	5.0 - 5.1	0.0173	0.9	16	4.85	4.7 - 5.0	0.0241	5.2
1	6	5.60	5.5 - 5.8	0.0519	2.3	16	5.38	4.4 - 5.8	0.0261	6.2
2	6 + 6	5.83	5.6 - 6.2	0.0489	2.9	15	5.73	5.0 - 6.6	0.0812	8.1
3	6 + 6	6.04	5.7 - 6.3	0.0436	1.7	17	6.24	5.4 - 8.1	0.0917	9.2
4	6 + 6	6.44	6.0 - 6.9	0.0877	4.7	18	5.97	5.5 - 6.3	0.0514	5.1
5	6 + 6	6.65	6.3 - 7.1	0.0755	3.9	17	6.62	5.9 - 7.3	0.0469	4.7
6	6 + 6	7.08	6.3 - 7.4	0.1141	5.6	15	6.27	6.1 - 6.5	0.0114	2.2
7	6	6.90	6.7 - 7.0	0.0520	1.8	18	6.32	6.0 - 6.8	0.0538	3.6
8	6	7.50	7.3 - 7.6	0.0520	1.7	15	6.89	6.5 - 7.2	0.1019	10.2
9	6	7.92	7.7 - 8.1	0.0609	1.9	15	7.09	6.7 - 7.5	0.0583	3.2
10	6 + 6	8.10	7.8 - 8.4	0.0510	2.2	19	7.41	6.5 - 8.1	0.0938	5.5
11	6	8.40	8.2 - 8.6	0.0775	2.3	16	7.72	7.3 - 8.0	0.0134	2.2
12	6	9.10	8.7 - 9.3	0.1000	2.7	15	8.06	7.2 - 8.4	0.0253	3.8
13	6	9.30	9.0 - 9.5	0.0448	1.2	17	8.31	7.8 - 8.9	0.0241	3.8
14	6 + 6	9.79	9.0 - 10.4	0.1591	5.6	15	7.57	7.1 - 8.1	0.0225	3.7
15	6	9.80	9.5 - 10.0	0.0933	2.3	18	7.91	7.4 - 8.5	0.0275	4.7
16	6	10.40	10.2 - 10.6	0.0633	1.5	13	8.99	8.6 - 9.6	0.1081	4.3
17	6 + 6	10.38	9.4 - 11.6	0.2433	8.1	15	8.81	8.3 - 9.5	0.0889	3.9
18	6 + 6	10.85	10.1 - 11.3	0.0959	3.1	16	9.22	8.1 - 9.9	0.3225	4.3
19	6 + 6	10.74	10.0 - 11.7	0.1573	5.1	13	9.25	8.9 - 9.5	0.0182	2.2
20	6 + 6	10.64	9.7 - 11.2	0.2088	6.8	14	9.49	9.1 - 10.4	0.0310	3.9

TABLE 10

SECOND SAMPLE

LENGTH ANALYSIS (Cms.)

Age in Days	SMALL LITTERS					LARGE LITTERS				
	Number	Mean	Range	S.E. of Mean	Coeff of Var. %	Number	Mean	Range	S.E. of Mean	Coeff of Var. %
0	6	5.02	5.0 - 5.1	0.0173	0.9	16	4.85	4.7 - 5.0	0.0241	5.2
1	6 + 6	5.42	5.2 - 5.8	0.0539	3.4	17	5.25	5.0 - 5.6	0.0458	3.6
2	6 + 6	5.78	5.3 - 6.2	0.0894	5.4	16	5.56	5.3 - 5.8	0.0458	3.3
3	6 + 6	5.92	5.4 - 6.9	0.1277	7.5	17	5.89	5.6 - 6.3	0.0500	3.5
4	6 + 6	6.93	6.6 - 7.2	0.0632	3.1	15	6.81	6.1 - 7.4	0.0794	4.5
5	6 + 6	7.23	6.7 - 8.0	0.1132	5.4	15	7.05	6.3 - 7.5	0.0894	4.9
6	6 + 6	7.62	7.0 - 8.0	0.1153	5.2	15	7.46	6.9 - 8.0	0.0831	4.3
7	6 + 6	8.13	7.9 - 8.4	0.0469	2.0	15	7.55	7.0 - 8.0	0.0693	3.6
8	6 + 6	7.75	7.6 - 8.0	0.0361	1.6	15	7.61	5.9 - 8.5	0.1536	7.8
9	6 + 6	8.55	8.1 - 9.1	0.0943	3.8	14	7.91	7.4 - 8.5	0.0800	3.8
10	6 + 6	8.69	8.1 - 9.2	0.1025	4.1	13	7.94	7.5 - 8.3	0.0648	2.9
11	6 + 6	9.05	8.7 - 9.3	0.0632	2.4	16	8.55	7.8 - 9.0	0.0800	3.8
12	6 + 6	9.57	9.3 - 9.9	0.0510	1.9	13	8.52	8.1 - 9.0	0.0721	3.1
13	6 + 6	9.92	9.5 - 10.8	0.0990	3.5	16	8.89	8.2 - 9.4	0.0889	4.0
14	6 + 6	10.02	9.3 - 10.7	0.1319	4.6	13	9.18	8.5 - 9.7	0.0927	3.6
15	6 + 6	10.68	10.2 - 11.4	0.1054	3.4	18	8.71	7.9 - 9.4	0.0985	4.8
16	6 + 6	10.55	9.9 - 11.4	0.1229	4.0	15	9.58	8.9 - 10.1	0.0819	3.3
17	6 + 6	11.53	11.0 - 12.0	0.0883	2.7	15	9.71	9.0 - 10.4	0.0954	3.8
18	6 + 6	11.43	10.9 - 11.9	0.0906	2.8	15	9.77	9.2 - 10.3	0.0748	3.0
19	6 + 6	11.48	11.0 - 12.4	0.1200	3.6	14	9.84	9.2 - 10.5	0.1105	4.2
20	6 + 6	12.12	11.2 - 12.7	0.1342	3.8	14	10.59	10.0 - 11.0	0.0911	3.2

TABLE 11

FIRST SAMPLE

GROWTH SUMMARY

Age in Days	WEIGHT GAIN (Grms.)		LENGTH GAIN (Cms.)		OBESITY INDEX		PONDERAL INDEX	
	Small Litter	Large Litter	Small Litter	Large Litter	Small Litter	Large Litter	Small Litter	Large Litter
0	-	-	-	-	0.2393	0.2470	2.737	2.756
1	0.95	0.10	0.58	0.53	0.2258	0.2387	2.904	2.986
2	0.86	0.18	0.23	0.35	0.2307	0.1855	2.936	3.138
3	1.09	1.68	0.21	0.51	0.2448	0.2008	2.911	3.141
4	1.09	0.16	0.40	- 0.27	0.2416	0.2253	2.987	2.985
5	0.77	0.42	0.21	0.65	0.2440	0.1928	3.009	3.253
6	2.24	2.25	0.43	- 0.35	0.2599	0.2722	2.857	2.842
7	0.70	- 0.62	- 0.18	0.05	0.2884	0.2524	2.881	2.926
8	3.47	1.29	0.60	0.57	0.3058	0.2395	2.899	3.067
9	3.55	- 0.62	0.42	0.20	0.3308	0.2139	2.881	3.217
10	0.79	3.90	0.18	0.32	0.3283	0.2668	2.931	3.038
11	2.38	0.54	0.30	0.31	0.3390	0.2549	2.915	3.118
12	6.53	1.75	0.70	0.34	0.3677	0.2608	2.915	3.141
13	0.47	3.87	0.20	0.25	0.3375	0.2990	2.952	3.208
14	1.32	- 7.06	0.49	- 0.74	0.3364	0.2399	3.075	3.161
15	1.28	2.76	0.01	0.34	0.3594	0.2639	3.010	3.104
16	3.11	6.35	0.60	1.08	0.3479	0.2829	3.103	3.170
17	2.54	- 0.65	- 0.02	- 0.18	0.3805	0.2862	3.027	3.137
18	0.10	1.64	0.47	0.41	0.3421	0.2806	3.167	3.204
19	3.31	- 0.36	- 0.11	0.03	0.3778	0.2745	3.052	3.228
20	0.62	0.23	- 0.10	0.24	0.3904	0.2745	3.009	3.258
Average per day	1.85	0.89	0.28	0.23	0.31	0.24	2.95	3.09

TABLE 12

SECOND SAMPLE

GROWTH SUMMARY

Age in Days	WEIGHT GAIN (Grms.)		LENGTH GAIN (Cms.)		OBESITY INDEX		PONDERAL INDEX	
	Small Litter	Large Litter	Small Litter	Large Litter	Small Litter	Large Litter	Small Litter	Large Litter
0	-	-	-	-	0.2393	0.2470	2.737	2.756
1	- 0.22	- 0.60	0.40	0.40	0.1978	0.1890	3.016	3.029
2	0.97	0.77	0.36	0.31	0.2029	0.1934	3.059	3.063
3	0.95	0.82	0.14	0.33	0.2206	0.1960	3.002	3.110
4	3.69	1.75	1.01	0.92	0.2378	0.1844	3.085	3.346
5	1.59	2.71	0.30	0.24	0.2489	0.2265	3.078	3.156
6	1.72	0.65	0.39	0.41	0.2537	0.2140	3.124	3.270
7	4.40	1.93	0.51	0.09	0.2894	0.2428	3.041	3.153
8	- 2.43	0.45	- 0.38	0.06	0.2780	0.2468	3.034	3.152
9	3.19	0.82	0.80	0.30	0.2721	0.2415	3.161	3.203
10	3.39	0.72	0.14	0.03	0.3083	0.2511	3.045	3.166
11	2.13	2.32	0.36	0.61	0.3102	0.2483	3.079	3.478
12	2.51	2.73	0.52	- 0.03	0.3049	0.2876	3.155	3.093
13	4.46	- 0.22	0.35	0.37	0.3290	0.2614	3.111	3.245
14	2.22	4.56	0.10	0.29	0.3446	0.2993	3.076	3.132
15	5.33	- 5.50	0.66	- 0.47	0.3501	0.2599	3.124	3.230
16	- 1.24	7.41	- 0.13	0.87	0.3476	0.2956	3.121	3.191
17	7.91	0.67	0.98	0.13	0.3505	0.2949	3.206	3.208
18	- 2.90	- 0.95	- 0.10	0.06	0.3351	0.2813	3.250	3.266
19	0.50	- 0.95	0.05	0.07	0.3360	0.2675	3.270	3.331
20	7.70	4.21	0.64	0.75	0.3539	0.2685	3.253	3.407
Average per day	2.29	1.22	0.36	0.29	0.29	0.25	3.10	3.19

Points of interest which emerge from the various analyses show clearly that in the two samples, both having been bred several years apart, the small litters have a much greater growth rate than the large litters. The differences were significant in both samples:

1) Regression lines calculated for length for small litters did not lie parallel since $P < 1\%$.
2) Similarly, calculations made for weight of small litters produced lines which were not parallel since $P < 1\%$.
3) In large litters calculations of regression lines for length indicated that they were probably parallel since $P < 5\%$.
4) Finally, calculations of regression lines for weight of large litters were not found to show parallelism since $P < 1\%$.

These differences can be attributed to the different samples of Sprague-Dawley strain used in the sequential experiments. Thus the maternal environment and the genetic endowment of the small litters differed significantly. The factors controlling the large litters consisted not only of the maternal environment and genetic endowment, but also a difference in the numbers presented in each sample, i.e. Sample 1 contained 333 rats while Sample 2 contained 317 rats.

Linear regression lines constructed for length (fig. 27) for both samples of small litters, clearly broke at two points - days 5 and 17. Length in the large litters (fig. 28) also broke on day 5 but showed a sample variation in their second break which occurred on day 14 (Sample 1) and day 15 (Sample 2).

Construction of regression lines for weight (figs. 29 and 30) for both samples showed that the previous pattern observed in length was followed by a break at day 5. The second regression line break occurred on day 14 which is earlier than those of the small litters. Since weight is more responsive to changes of nutrition than length, the earlier break of the regression line on

Figure 27 Regression line construction for length growth of control (small) litters of Samples 1 and 2 showing the triphasic pattern achieved over 20 days. The calculations were based on the breakdown of the total regression line by means of the largest overall difference of the regression coefficient "b". The breakage points in both samples occurred on day 5 and day 17 (Sample 1 contained 333 rats while Sample 2 contained 317 rats).

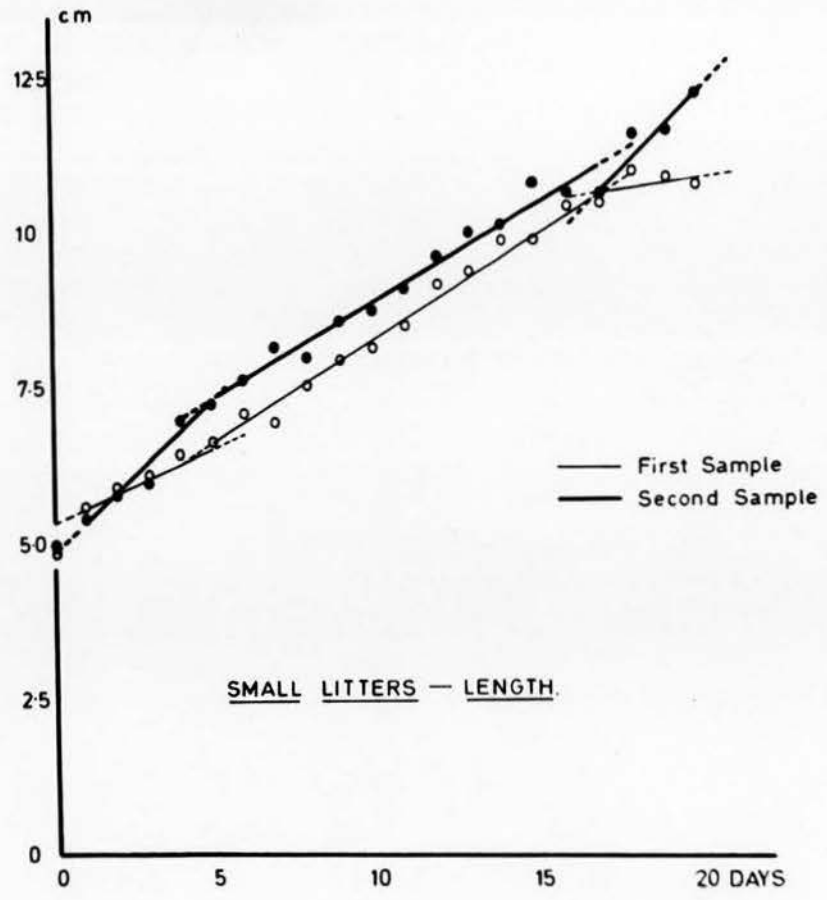

cm
12·5
10
7·5
5·0
2·5
0
0
5
10
15
20 DAYS
First Sample
Second Sample
SMALL LITTERS — LENGTH.

Figure 28 Regression line construction for length growth of large litters derived from Samples 1 and 2 showing the triphasic pattern achieved over a 20 day period. The breakage points of the regression lines were calculated from the regression coefficient "b". Note that the first phase of the triphasic pattern terminates on day 5 in both samples, whereas the second breakage point differs by one day in the samples. This difference can be regarded as within the range of litter variation.

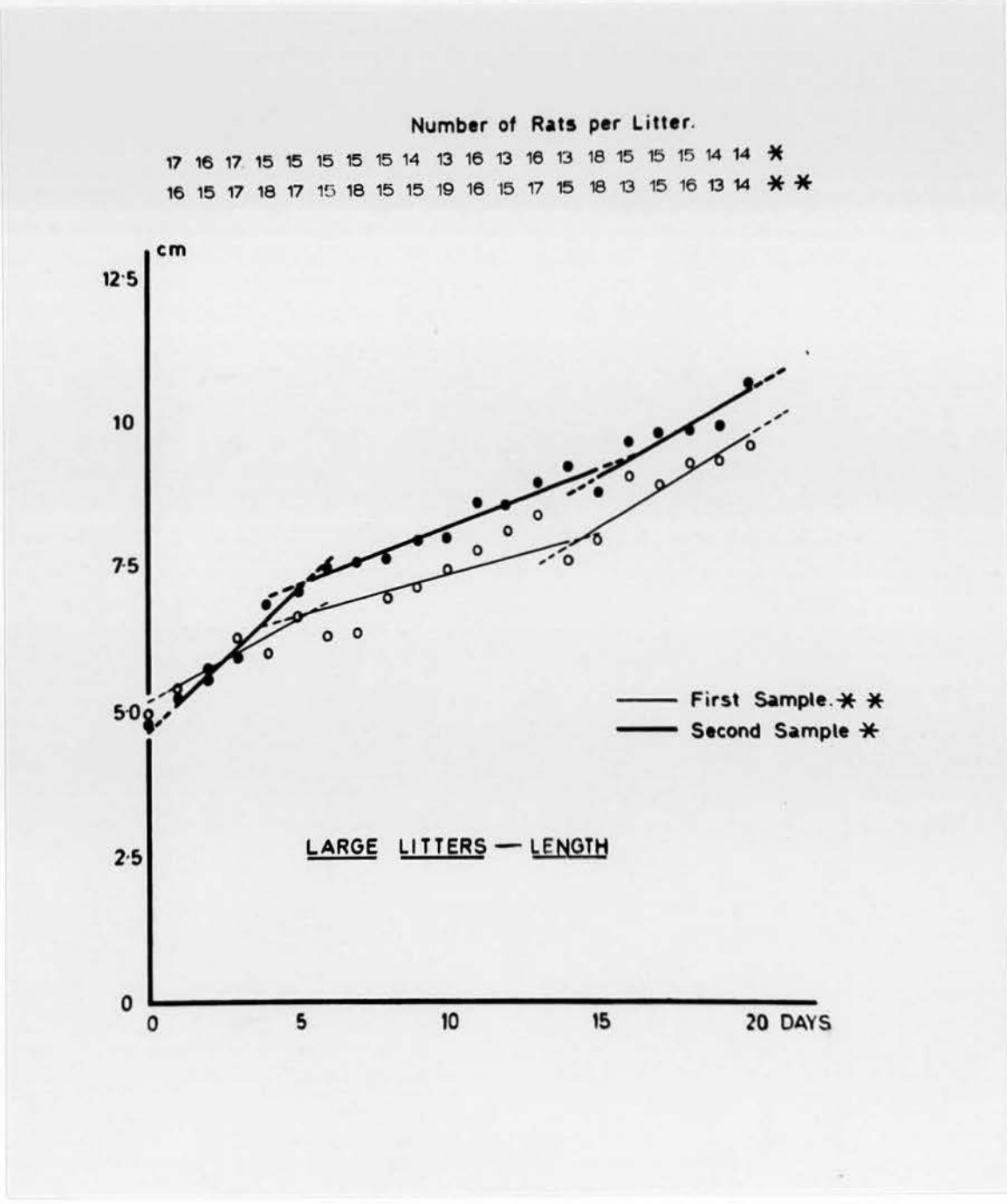

Number of Rats per Litter.
17 16 17 15 15 15 15 15 14 13 16 13 16 13 18 15 15 15 14 14 *
16 15 17 18 17 15 18 15 15 19 16 15 17 15 18 13 15 16 13 14 * *
cm
12·5
10
7·5
5·0
2·5
0
0
5
10
15
20 DAYS
First Sample. * *
Second Sample *
LARGE LITTERS — LENGTH

Figure 29 Regression line construction for growth by weight of control (small) litters from Samples 1 and 2 showing the triphasic pattern outline. The first breakage point of the regression lines occurred on day 5. The second breakage point of Sample 1 occurred on day 17 while that of Sample 2 was two days earlier - day 15. Weight, as a measurement of growth, is invariably more sensitive to environmental changes and litter variance.

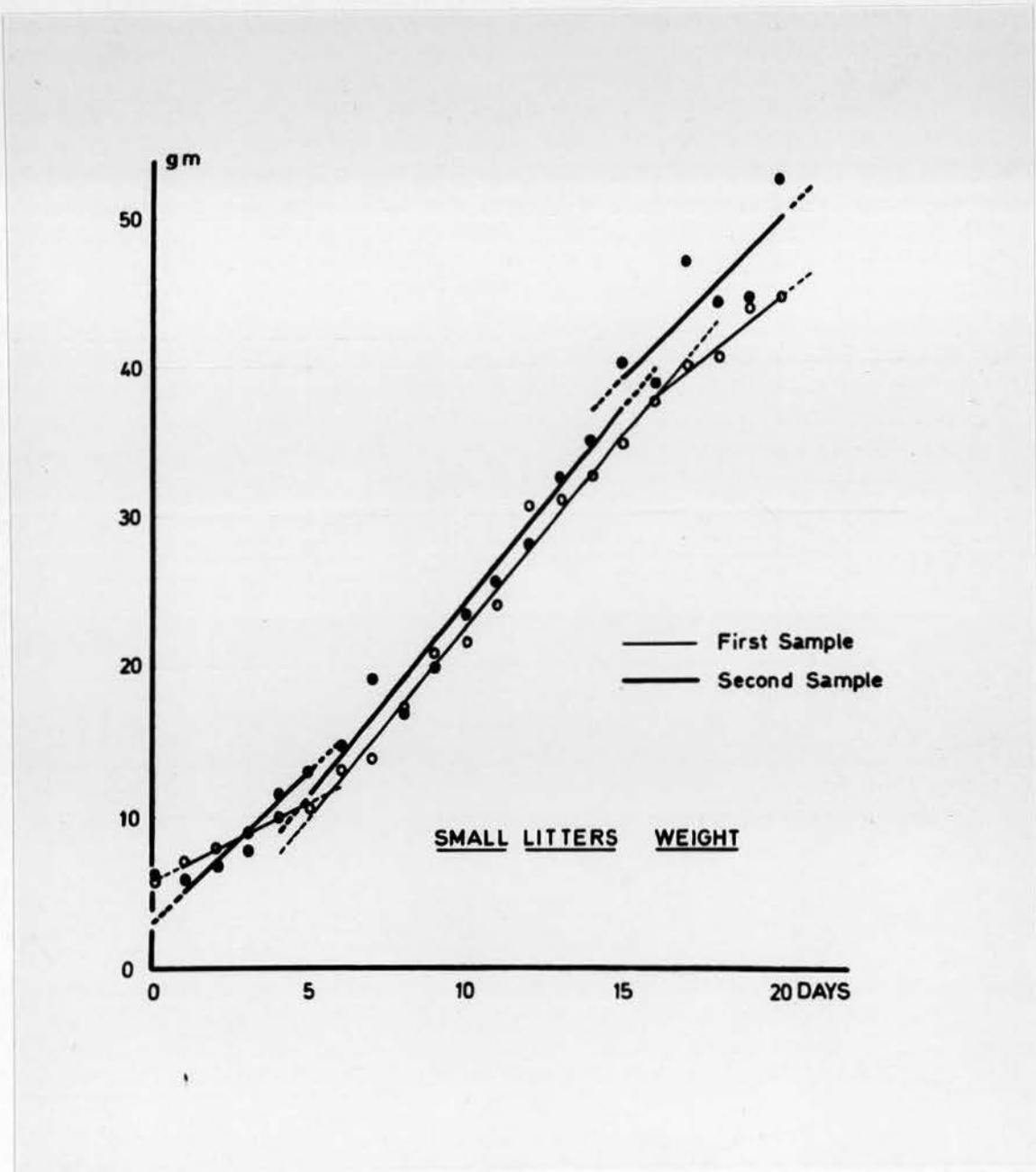
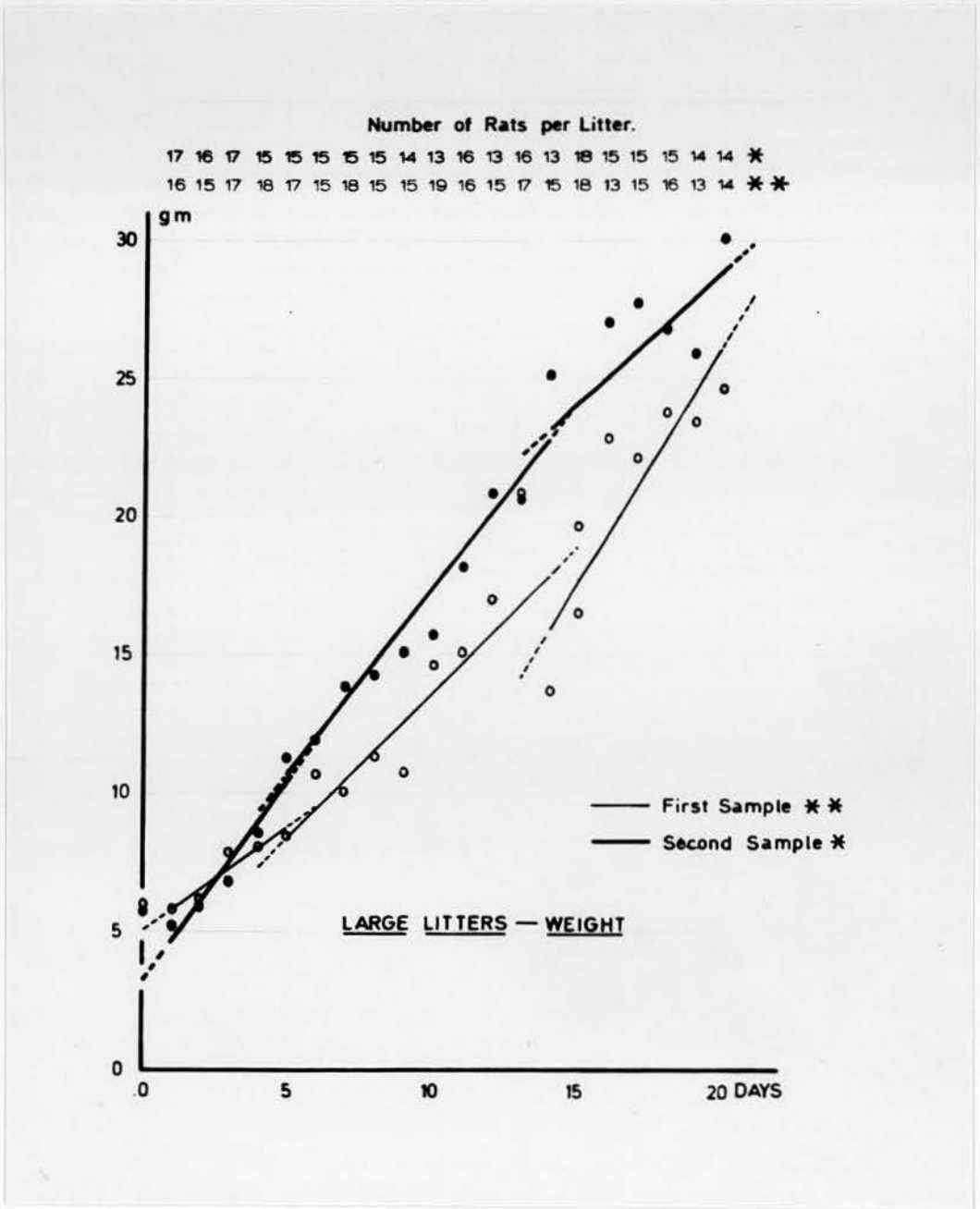

gm
50
40
30
20
10
0
0
5
10
15
20 DAYS
First Sample
Second Sample
SMALL LITTERS WEIGHT

Figure 30 Regression line construction for growth by weight of the large litters from Samples 1 and 2 showing the triphasic pattern outline. Both Samples demonstrate the first breakage point on day 5, with the second breakage point on day 14. When these second breakage points are compared with those in Figure 29, it will be seen that the large litters must have found a new source of energy - probably the intake of solid food and therefore an earlier weaning.


Number of Rats per Litter.
17 16 17 15 15 15 15 15 14 13 16 13 16 13 18 15 15 15 14 14 *
16 15 17 18 17 15 18 15 15 19 16 15 17 15 18 13 15 16 13 14 **
gm
30
25
20
15
10
5
0
0
5
10
15
20 DAYS
First Sample **
Second Sample *
LARGE LITTERS — WEIGHT

day 14 of the large litters can be construed as evidence of an early supplementation of the declining milk supply by solid food intake. Although the particular day upon which the second break occurs is not consistent for length and weight data, it must be remembered that the amount of change exhibited by length and weight, when small and large litters are compared (Tables 6, 9, 10) show considerable differences of which weight appears to be the more sensitive to various environmental factors. Hence the particular day of breaking may simply reflect inherent variation of the maternal environment (maternal capacity) and, to some degree, the genetic endowment. Examination of the data of both samples shows that the large litters are more heterogeneous than the small litters.

4.4 Patterns of Body Growth - Preweaning Discussion

The criterion of sex difference based on body size - males being accepted as being heavier and longer than females - has been used successfully on most adult mammals. The subject in relation to the present observations has been touched on (Materials and Methods, Section 2.6 and Appendix) previously but has come forward again for further comment prior to discussing details of the growth patterns.

Any trend of division by weight or length of sex within the preweaning period is going to be a difficult point to prove against a background of environmental influences of all kinds, and the genetic endowment. Very often one can pick out a rat from a litter and discover that it is heavier than most and also a male. However, it is equally possible to select another heavy rat and find that it is a female. Examination of previous work shows that the weight of a rat at birth was used by KING (1915) and that his observation that the males were heavier was later supported by ANGERVALL (1959). During the final stages of the preweaning period ACHESON et al (1959) noted that the males were heavier. In refutation of these observations, LINDH (1961) clearly showed that there was no evidence of

sexual division in the prenatal weight of rats - a point which suggests that differences of weight observed at birth are more by chance than evidence of a sexual difference. A special study was made by SWANSON and VAN der WERFF ten BOSCH (1963) and they could not find any significant evidence of sex difference in young rats until they had reached some 30 days old. Following on from 30 days there was a marked divergency of both the ponderal and linear growth curves. This has now been supported by the observations of KIDWELL et al (1960), ROUBICEK et al (1964) and the recent work of PARK (1970). Within the present data there are no significant differences which warrant any division of the sexes and for purposes of examining the preweaning changes the analyses have been conducted on amalgamated males and females. For purposes of reference, however, it was decided to display the data in such a manner that if and when information is required from either sex the relevant figures should be available.*

The complexity and sensitivity of the responsiveness of various species of rodent to environmental manipulations have arisen from the work of SCHNEIRA (1959) and RHEINGOLD (1963) and involved the relationship between specified early experiences and physiology together with the maternal and infant interactions. One example of environmental manipulation is the culling of litters so that they are formed only of one sex. This particular practice can be used for studies involving castration or gonadal hormone administration.

From these particular procedures emerged some new insights into weight variation based on the work of BRAIN and GRIFFIN (1970). They were analysing some control litters when they noted that body weights taken at weaning showed that males and females in litters consisting of littermates of the same sex were heavier than males and females, respectively, in litters of equal size, but of littermates of both sexes. These differences were observed to gradually level off over the following weeks. Since the preliminary observation indicated

*See Appendix A.

a relationship between the type or amount of behavioural interactions within the litter and the sex, the investigation was extended. Three types of litters were formulated: (1) a homogeneous-male litter, (2) a homogeneous-female litter, (3) litters with equal numbers of males and females. Results showed that males and females reared in litters formed only of members of the same sex were heavier than the males and females, respectively, brought up in litters of equal size, but containing both sexes. One of the interesting points relevant to the present work is that the differences were evident at day 14, in other words into the second phase (milk decreasing - solid food increasing). Although the tri-phasic growth pattern would not be altered by using only males or females, the continuity of the litters and the variance of weight in a normally mixed litter was deemed more appropriate to the experimental philosophy than the separation of litters by sex alone to gain some slight weight increase.

Variation in weight and size of preweaning rats is generally accepted as varying inversely with the number of litter-mates in competition for the available milk and the evidence certainly suggests that the controlling overall factor is one of litter size versus the maternal capacity. There are a number of growth changes within the preweaning period which when combined could have some effect on a growth curve. These changes include the functional emergence of various sensory organs and development of the masticatory system. For example, the ears of a young rat open around day 5, the eruption of the incisors ranges between 5 to 7 days, the opening of the eyes usually occurs around day 12 and with it increased movement of the animal. The first molars begin to erupt followed approximately 2 days later by the second molars - so that between 13 to 18 days a total of 8 mandibular and maxillary teeth have emerged or are emerging. A further sign of significance in relation to possible feeding habits is the signs of abrasion on the tips of the incisor teeth somewhere between

15-17 days.

Arising from these changes the question might well be asked - "What effect would the introduction of the auditory and visual systems have on the growth curve and what is the consequence of tooth eruption?" The answer is that they do not have simple individual direct effects but rather their effects are cumulative. For example, once vision is established where previously the young rat, once the mother had moved to the other end of the cage, would make a disorientated search for warmth and food, it now would be able to move in the correct direction. This in turn helps to promote mobility and so more energy is burned up in movement than previously. Auditory system will support the locatory mechanism of the visual. In the question of erupting teeth we are on thinner ground and can only postulate. If the human baby is anything to go by, then teething troubles are a well known phenomenon. In the rat the teeth are the full adult size and they are situated in an immature jaw structure, thus the possibility of the eruption of 8 adult molar teeth over a few days causing some form of disturbance is highly possible. The reason why it is impossible to isolate the effect (if any) is because the eruption timing falls within the timing of the decline of milk supply and the gradual intake of solid food.

Examination of Table 7 shows that the standard error and the coefficient of variation for both day 10 and 20 of the small control litters are greater compared to the others. One might regard day 10 as being representative of the maximum milk supply whose peak is reached within that period but this would be too easy an anwer since information of the lactational longevity and maximum are not known for these rats and therefore the idea of a milk peak must be regarded tentatively. The high figures obtained for day 20 can be linked to food supply since the process of biological weaning has been taking place since approximately day 12. However, another factor which begins to emerge is that the control of growth at

this point is by the pituitary gland and with it the genetic endowment takes over from the maternal environment. It is interesting to note that the weights of the rats taken during the last few days show a considerable range which could be attributed to varying individual capacities of solid food assimilation, the presence of litter-mate competition - a point of importance since the solid food source at this time is often the fragments of rat-cake which the adult rat has pulled down, and possible slow adaptation to the new food source. Tables 11 and 12 show the weight gain in the small control litters to be constant while length, although not so dramatic, shows the influence of litter variability. In the larger litters, neither gain in weight nor in length appear as consistent as those of the control litters. This problem will be returned to again a little later on.

The concept of successive growth cycles was introduced and developed by BRODY (1927, 1928, 1945). A review of the formulations of rat growth and growth cycles reported by other workers has been under by ZUCKER et al (1941a, b, 1942) who suggested that there were two primary phases of development in the rat - the preweaning and the postweaning. Originally GRAY (1928-29) had pointed the way by drawing attention to the lack of validity which followed theories of cyclic growth which were based on curves derived empirically by fitting sub-optimal growth data. ZUCKER et al (1941a, b, 1942) supported this contention and went on to say "if one accepted all of the different possible causes of cycles which have been suggested as definite evidence that all these cycles exist and must be considered, it would have to be concluded that growth is so complex that even an approximate valid quantitative formulation is out of the question ..... the greatest difficulty in attempts to formulate growth is to avoid letting the general concept of cycles degenerate into a mere adjunct to empirical curve fitting ..... at the opposite extreme, just as logical,

is the position that no cyclic analysis is fully justified, and therefore none must be made; the data must be fitted whole, or not at all ..... We feel that an intermediate position, just as logical, but more realistic, would recognise the possibility of cyclic growth but would demand more detailed and objective evidence in support of any particular cyclic scheme offered".

With the convergence of research methods towards a central theme, the aspects of their particular approach must be accepted since it is impossible for one researcher to follow more than one or two paths at one time, not only is there insufficient time but, more important, the expertise required is impossible to assimilate to the level required. Thus, on making some findings, it is only correct to "cast about" in other fields to ascertain if the "bumps" visible on a graph could have a very different interpretation. As will be gone into in detail during the discussion of the cranio-facial growth pattern, the endocrine control and other physiological factors have an important contribution in the growth spectrum seen in the body.

A number of additional growth phases to those stipulated by ZUCKER et al (1941 a, b, 1952) have emerged on the scene from, notably, DUNN, MURPHY and ROCKLAND (1947) in the postweaning period, and in the preweaning period by MURPHY and DUNN (1948), PARK (1969), HUGHES and TANNER (1970) and PARK and NOWSIELSKI-SLEPOWRON (1971, 1972a, b). It is only fair to add at this point that two of the more pertinent postulates relating to preweaning growth cycles emerged from BRODY (1927) and DAGGS (1935).

As advocated in the introduction of this thesis, the Gestalt approach has been used so that the growth of the whole animal has been examined before relating a part of the whole (the skull) to it, or for that matter the popular method of comparing the growth of a part to the growth of another part. A considerable amount of work has been done on various body parts such as the

kidney (STOERK and ZUCKER, 1946), femur ash (ZUCKER and ZUCKER, 1946), protein (ANDERSON and SMITH, 1940), fat (HATAI, 1917, IOB and SWANSON, 1938), collagen (NEUBERGER and SLACK, 1953) and the aspects of cell organisation and related patterns (PICKEN, 1960). The field stretches indefinitely covering water, calcium, iron, creatine, etc. and the quoting of all the sources will not further the discussion at this point. The important point emerging from these studies, especially those which have been investigated during the early development of the rat, is that many of them contain plots of their data against a time scale and that there are many of these plots which have either breaks or have indications where breaks might occur - some of these might be, in reality, part of a growth cycle. Many of these changes of direction of the plot or breaks can be linked with whole body growth and could easily be governed by the same controlling factors.

The problem of comparing data with that produced by others inevitably runs into snags of various kinds. For example, there exist wide discrepancies in empirical growth data as shown by the work of ZUCKER et al (1941a, b, 1942), DUNN, MURPHY and ROCKLAND (1947), MURPHY and DUNN (1948), MAYER (1948) and BERTALANFFY (1960). The well known data of DONALDSON (1924) was not considered to be satisfactory because the increased growth rate coupled with the change of shape of the growth curve was found to be obtainable when an improved laboratory diet was introduced. In short, each of the mentioned groups of investigators proposed a different equation for the growth of the rat, which had the supreme drawback of not being able to fit the data of others. Thus, on the surface it can be said that the growth of rats can show a modified and sometimes widely differing curve by the introduction of various diets. Although there are many other reasons such as errors of methodology - observer error, instrument error, environmental error and object error (PARK, 1970) -

the basic reason for having two separate samples of rats for investigating the body growth spectrum was to allow the variance between two separate populations to be clearly demarkated while living under identical external environmental conditions.

Examination of Figures 27, 28, 29 and 30 demonstrates the growth from birth to 20 days of the head-body length and weight of the small and large litters derived from two samples. In all these figures it will be noted that the first phase of growth terminated on the same day, i.e. day 5. Naturally, the two most important factors controlling this early growth phase are the maternal environment - regarded as the inherent capacity of the mother to rear her young, and represented in practice for this purpose as nutrition, and the genetic endowment. There are, of course, other controlling factors of growth, which unless they have undergone some maladjustment of their physiological mechanism, should not induce fluctuations of growth rate. Although these other factors will be discussed later, it suffices to point out that within the first phase of growth, i.e. birth to day 5, the general growth is accepted as autonomous.

At birth, the young rat undergoes a change from internal to external environment which includes a new way of life and type of food supply. Within the first few days, the lactational capacity of the mother increases with the stimulation of the mammary glands* by the young. The period during which suckling takes

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* The rat usually has six pairs of mammary glands, three thoracic, one abdominal, and two inguinal. The first pair of thoracic glands is located anterior to the forelimbs at the root of the neck and is just under the skin. The second and third pairs of thoracic glands spread out in the subcutaneous muscle and connective tissue over the sides of the thorax and extend into the axillae. The abdominal and inguinal glands lie in a thick fat pad on the ventral surface of the abdomen. Each mammary gland arises from a single main duct at the nipple and extends laterally by an irregular process of branching. As a point of interest, the mammary glands of the male rat have no nipples and the main duct ends blindly in the dermis at a point where a nipple would normally be found in the female. For embryonic and postnatal development of the rat mammary glands the reader is referred to MYERS (1916, 1917a, b, 1919) and ASTWOOD, GESCHICKTER and RAUSCH (1937).

place is characterised by an anabolic capacity never equalled later (CZAJKA-NARINS, MILLER and BROWNING, 1973). The anabolic tendency increases the dependency of the young rat on a regular intake of milk. Within the whole period of suckling there is an extensive increase in the size of the body, of various organs - although not all organs increase at the same rate at the same time, and various metabolic processes become controlled homeostatically by the emergence of endocrine control. If we take temperature as a typical example, the control of temperature is linked with brown fat deposits (one of the reasons why a human baby does not shiver!) during the initial stages, the chemical temperature regulation begins to act between 10 to 14 days (HAHN, KRECEK and KRECKOVA, 1956), and this is followed by the physical regulation from approximately day 14 to 18, for the next few days (postweaning period) both these regulators are present then from 25 days onward there is a greater deposition of subcutaneous fat so that by day 30 there exists an insulatory layer. At the same time as the subcutaneous fat is being laid down the development of the vasomotor system is completed. Thus the changes in the life of a developing rat appear to depend a great deal on an adequate supply of food and because of this a developing rat is vulnerable to changes in diet both quantitatively and qualitatively.

If we return to the first phase of growth (birth to day 5), we must remind ourselves that weight is more sensitive to change than length so that changes in weight (outside the range of normal variance) will be observed first. Within the five days following birth there is a slow build-up of milk supply due to the stimulation required by the mother. In the large litter there is a possibility that the greater stimulation will produce more milk relative to that of the small litter, this however, is of course offset by the greater number of mouths for feeding. It is difficult to separate the two types of litters in the first two days since there is variance in initial birth weights to be taken into account. However, a trend does emerge in the weights of the large litter which

by day 5 has become much more noticeable. One can postulate that day 5 probably represents the normal milk producing capacity of the mother beyond which only a small percentage of change can be expected. Thus within the first phase we have the readjustment of the young to a different source and type of food, an adjustment of the maternal behaviour, an initiation of lactation with a gradual increase to full capacity with the aid of mammary stimulation from the young and a general autonomous growth control. Weight, as expected, shows the greater fluctuation but generally the first 5 days confirms that irrespective of the level of nutrition that it is a period of rapid growth which in relative terms is large when all the problems of change are considered.

The second phase of growth continues from day 5 to day 17 in the head-body length results of the small litters in both samples. In the large litters, although there is a difference of 1 day between the samples, i.e. the regression line breaks occurring at days 14 and 15, the overall picture is one of an earlier change of growth pattern than that of the small litters. Examination of the lines constructed for weight shows a greater difference in the times of the breaking points of the lines of the small litters, Sample 1 breaking at day 17 while Sample 2 breaks at day 15. Interestingly enough, the large litters have no such discrepancies since both samples break on day 14.

This second phase covers the time when the lactational capacity of the mother is thought to reach its maximum level somewhere around 12 to 14 days although there are indications that it may be as early as 10 days. The exact point is not of significance in the present observations since even with a range of 10 to 14 days it still occupies the central sector of the second phase. The question of the use of the word "maximum" must be related to the fact that the quantity may not have exceeded the 5 day level by more than a few per cent - if any change has occurred - and we are really taking the period of birth to day 5

as the "starting" phase followed by the "full production" phase. It must also be remembered that apart from individual variables, those mothers supporting large litters will have received greater mammary gland stimulation hence will have a different "maximum" lactational capacity compared with those with small litters - even though the growth results are directly altered through the greater demand of a raised but still insufficient supply. Within the second phase lies the point where the amount of milk begins to decline and it is interesting to note that there is no reference in the literature regarding the qualitative state of this milk.

The differences within the small litters can be postulated as part of "litter variance", i.e. the inherent differences normally existing within the range of the maternal environment and the genetic endowment. The larger litters exert greater demands on the mother thus one would expect the steady growth trend to be lengthened in the sense of retardation. What is observed, however, is an earlier start of the third phase in the large litters, which can only mean one thing - a new source of energy. The only source of energy present is that obtainable from the solid food supply of the mother which is usually situated at one end of the cage. I have never actually observed a mother rat pull rat cake down for her offspring but this does not rule out the possibility. Normally there are a number of fragments on the floor of the cage and the more agile young could reach upwards for other pieces. Let us accept that the maximum milk supply has been attained and is now dropping, in the large litters this peak is obviously reached a little earlier through the greater demands hence a greater drive is generated in the large litter members to find more food. Evidence comes in the form of incisor tooth wear which appears in the small litters around day 16 and in the large litters a little earlier. It must be stressed that an observation may be noted at a definite day but that a day is a considerable length of time in

the life of a rat and that in reality the changes are not sudden in nature but are probably gradual relative to the rat. The maxillary and mandibular incisor teeth exhibited facets of abrasion which would take a little time to appear hence the possibility of an intake of rat cake fragments prior to tooth wear. One argument which can be aligned against this postulate is that since they interlock for the purpose of gnawing that the wear could have arisen through natural "bruxism". While this is acceptable by itself, it does not answer the question of why the small litters exhibit facets of wear later than the large litters - unless hunger stimulates "bruxism"!

As a tentative follow-up to the wearing of incisor teeth, the stomach contents of several young rats were examined by means of an ordinary light microscope and fragments of semi-digested rat cake was noted prior to wear appearing. A properly conducted stomach content analysis was not possible through the lack of technical assistance, nor was a faeces test possible.

The second phase of growth is significant for another reason - within its boundaries lies the true biological weaning point. Weaning from an animal breeder's point of view is when the young can be removed without damage to their growth and being able to accept substitutes in the form of cow's milk, etc. In the laboratory world the age of weaning of mice and rats differs but is usually acceptable at 20-25 days. Naturally, most young animals will go on feeding on milk as long as it is available, so it is obvious that weaning is a gradual process which makes its presence felt by the need for extra food supplies. The commencing point of biological weaning must be the decline of milk supply - which as already stated ranges between 10 to 14 days - by the time the wearing of facets on the tips of the incisors has appeared, the process has obviously moved sufficiently along its path to have stimulated the young rat to make efforts for a new source of food. With the large litters exhibiting incisor wear earlier

than the small litters, it can only be presumed that the biological weaning point has been reached a little earlier. What is not known, however, is whether the maximum lactational capacity, which is raised in level by the larger litter, comes to its peak earlier and then falls, or reaches its peak at the normal time but cannot sustain its natural fall at the rate accomplished by a mother of a small litter.

A further complication stems from the overall growth control of the rat. So far we have dealt with the effects of the maternal environment (covering the female's nursing ability, lactational capacity, etc.), the external environment (this increases as the maternal influence falls and covers such items as solid food, warmth, nesting conditions, etc.), the genetic endowment (which has very little influence at the start but increases as the maternal environment decreases - see Introduction, Section 4.1). All these factors have effects on the rise and fall of the growth pattern but within a limited range. The direction of the growth of the animal during the first phase (birth to day 5) is autonomous (JENKIN, 1970), in the second phase (day 5 to 17) the control is beginning to alter, still autonomous, but with early pituitary factors emerging. The rate of growth will alter, the quality of growth will change, the quantity - in the sense of mass - will alter, but the essential direction is maintained as long as there is enough energy to give it momentum.

Phase three covers the last period of the so-called preweaning zone (birth to 20 days) and appears to be short since it only lasts some 5 days depending on the litter-size. In reality the third phase continues into the next main division accepted in a rough classification as the postweaning period. However, since we have employed the divisions of preweaning and postweaning as a simple yardstick of change, they will be continued as a useful way of orientation until a firmer basis for selection can be devised. During this phase the main source

of food is rat cake although any milk available would be quickly consumed. Growth rates in all the litters show a rapid upward surge by the steepness of the regression lines. The growth within phase three lies mainly under the influence of the external environment, the genetic endowment and the control of the pituitary hormones.

The triphasic growth pattern of the rat body by weight and length has been demonstrated clearly during the first 20 days following birth and the variance in the breaking points of the regression lines appears to stem from some interference in the maternal environmental pattern. Variance with a normal range covers comparisons of small litters but the variance observed between large and small litters is obviously accentuated by the stress placed on the mother of the large litter. The growth of these young rats during the main period of maternal environmental control (the lactational period) is influenced by their own genes, as well as by environmental influences, and a portion of these may be attributable to the genotype of the mother. As LEGATES (1972) pointed out, the major difficulty is in attempting to separate the transmitted and direct effects of the mother on the growth of her young. The mother transmits a sample half of her genes to each member of her family and her genotype for maternal effects is also influencing the postnatal conditions of their growth.

LEGATES (1972) made some distinction between the use of the terms "Maternal effects" and "Maternal influences" and this has proved a valuable addition for discussional use. "Maternal effects" are regarded as the measured phenotypic expressions arising from influences of the mother on a trait measured in her offspring, apart from the direct influence of the genes she transmits. The other side is dealt with by the "Maternal influences" which refer to those things which condition the expression of the maternal effects. Taken from the mother's side, these result from her genotype and related environmental factors whereas, taken

from the young's side, the maternal influences may be considered as environmental in nature, conditioning the expression of the offsprings' genotype for the trait measured.

Within the maternal influences playing on the large and small litters there are undoubtedly factors such as temperature, maternal instincts, maternal experience, etc. but the lactational capacity appears without doubt to have the major influence. Various postnatal effects have been investigated in rats and mice (BATEMAN, 1954; YOUNG, LEGATES and FARTHING, 1965; EL OKSH, SUTHERLAND and WILLIAMS, 1967; BLUNN, 1969; EISEN, 1970; EISEN, LEGATES and ROBISON, 1970; HANRAHAN and EISEN, 1970; NAGI, 1971; LEGATES, 1972) and it is now generally accepted that maternal effects play an important role in the early growth of both rats and mice, and in relation to the present work to the First and Second preweaning phases of growth. These workers noted that the influence diminishes after weaning, which in the present observations must lie between 15 and 17 days, and that growth determination by the genes of the young increases, i.e. in the Third phase. It can be postulated, therefore, that the use of a large litter brings the point of biological weaning a little earlier and in so doing allows the gene determination of growth to occur a little faster than usual.

Maternal effects introduce important complications to any study of size or growth, especially when linked with inheritance since they can be a major source of resemblance between maternal relatives and their effects may confuse with resemblance resulting from genetic similarities between those relatives. This particular aspect is, of course, the main source upon which quantitative genetic theory is based, and to encourage any advancement in the subject, understanding of the nature and magnitude of the maternal influence has become paramount - the emergence of a triphasic growth pattern within the preweaning period (birth to 20 days) may extend this understanding a little.

4.5 Patterns of Body Growth - Preweaning Summary

Body growth of two separate samples of rats formed into small and large litters by cross-fostering technique was examined over a period of 20 days (birth to 20) at 24-hourly intervals. The basis of the larger litters was to obtain a reduction of the amount of milk available to the young rats by over-taxing the mother's milk producing capacity. The pattern of body growth studied by means of weight and head-body length revealed a characteristic triphasic spectrum in the measurements of both sizes of litter and from both samples. Linear regression analysis showed that the First phase covered the period of birth to day 5, while the Second phase covered a period of 5 to day 17 in the head-body length of both samples of small litters. The large litters showed a slight difference of 1 day between the samples, i.e. day 5 to 14 and day 5 to 15. Examination of weight showed the small litters to have phases of 5 to 17 days (Sample 1) and 5 to 15 days (Sample 2) whereas the large litters showed uniformity having a Second phase period of 5 to 14 days in both samples. The Third phase starting point depended on where the Second phase had ended but regression line analysis indicated a different growth rate up to the final day (20). The triphasic spectrum stems from the interplay of the external environment and the genetic endowment. Variables implicit in the concept of "external (maternal) environment" have been represented by those of "nutrition". The control exerted by these two factors on the growth have been confirmed by experimentation of litter-size.

4.6 Patterns of Body Growth - Postweaning Methodology

The large and small litters used over the postweaning period of 20 to 40 days stemmed from the same breeding system (Materials and Methods, Section 2.4), i.e. the random selection of young at birth and the use of the cross-fostering technique of KENNEDY (1957). The series was examined at 24-hourly intervals,

killed by an overdose of ether before being weighed and measured. The heads were removed for later preparation and measurement. The main difference between rats examined from the pre- and postweaning stages was that in the postweaning stage a sexual division was established from the beginning. All biometric analysis has therefore been worked out under the titles of "male" and "female". The next difference lies in the presentation of the data - apart from the use of tables showing the basic and analysed results. Linear regression line analysis did not provide any advantages over the simpler methods and so after close inspection of the data it was decided that the recovery or non-recovery of the large litters compared to the small litters could be very well expressed by means of distribution charts - Figures 31, 32, 33 and 34.

4.7 Patterns of Body Growth - Postweaning Observations

Several points of interest emerge from the distribution charts and the Growth Survey Tables (the latter to be found at the end of this Chapter).

When weight is inspected in the small litters, the appearance of a division by sex is slow to finalise since the amount of difference by weight is probably well within the confines of the individual variance. The mean-weight calculations of males can be set out as follows beginning with day 21 to day 29:-

55.42g, 39.67g, 53.55g, 58.28g, 61.50g, 84.63g, 74.90g, 73.0g, 79.33g.

Similarly, the corresponding female mean-weight can be arranged thus:-

53.14g, 38.20g, 53.88g, 55.20g, 60.80g, 80.37g, 69.36g, 75.77g, 92.90g.

It will be noted that where a male returns a high weight that the weight of the corresponding female mean weight is close to it. Since the males and females are members of the same family group, i.e. under the same maternal environment during their preweaning phase of life, it appears that they all started with slight differences of weight at birth and that these differences had been maintained uniformly throughout pre- and postweaning phases up to 29 days. Most of

Figure 31 Distribution graph plotted on the means of the small litter weights of both males and females and covering the period of 20 to 40 days.

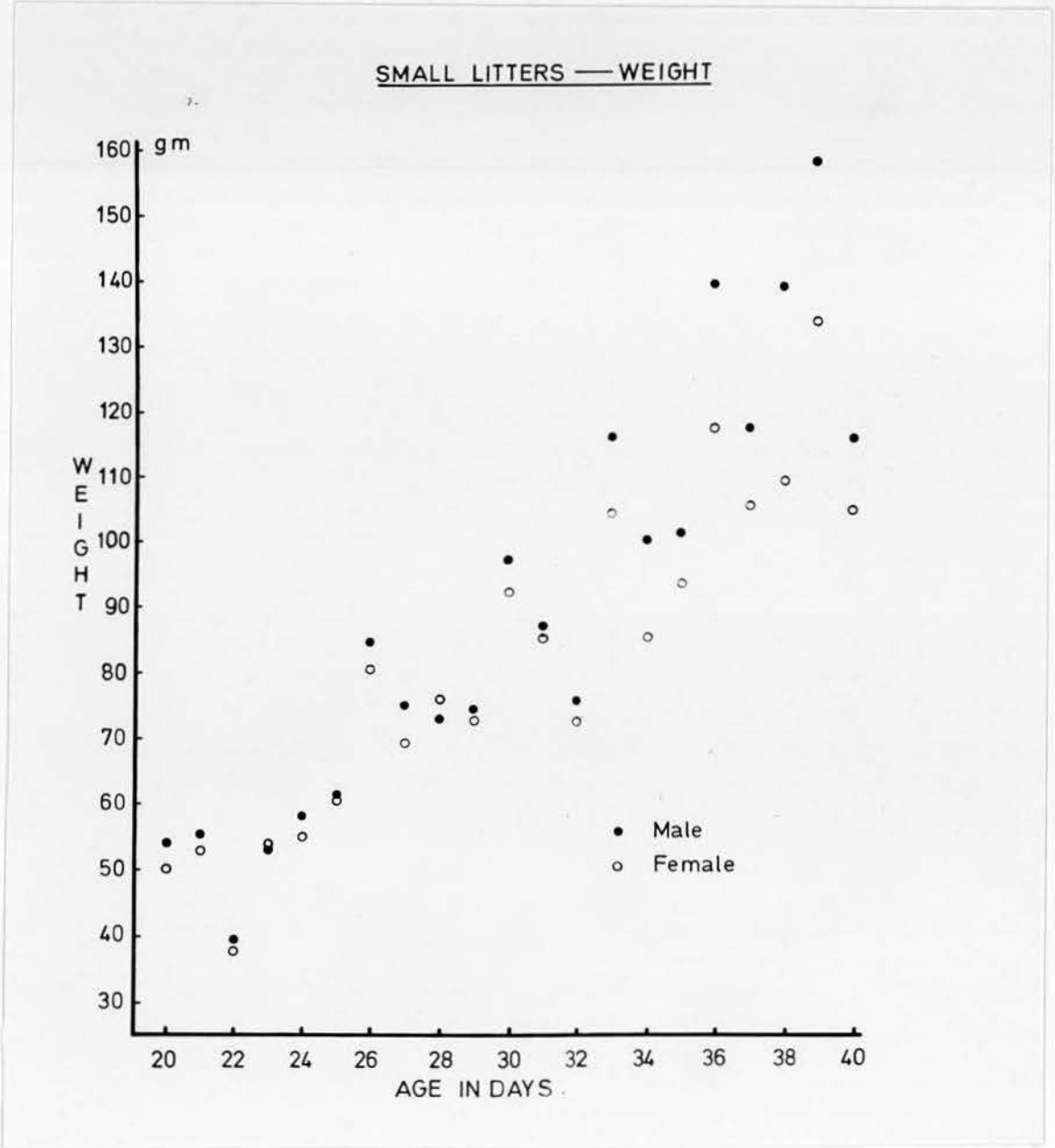

SMALL LITTERS — WEIGHT
gm
160
150
140
130
120
110
100
90
80
70
60
50
40
30
WEIGHT
20
22
24
26
28
30
32
34
36
38
40
AGE IN DAYS
Male
Female

Figure 32 Distribution graph plotted on the means of the large litter weights of both males and females and covering the period of 20 to 40 days. Compare with Figure 31 and note that large litter mean weight of neither male nor female reach above the 100 g mark.

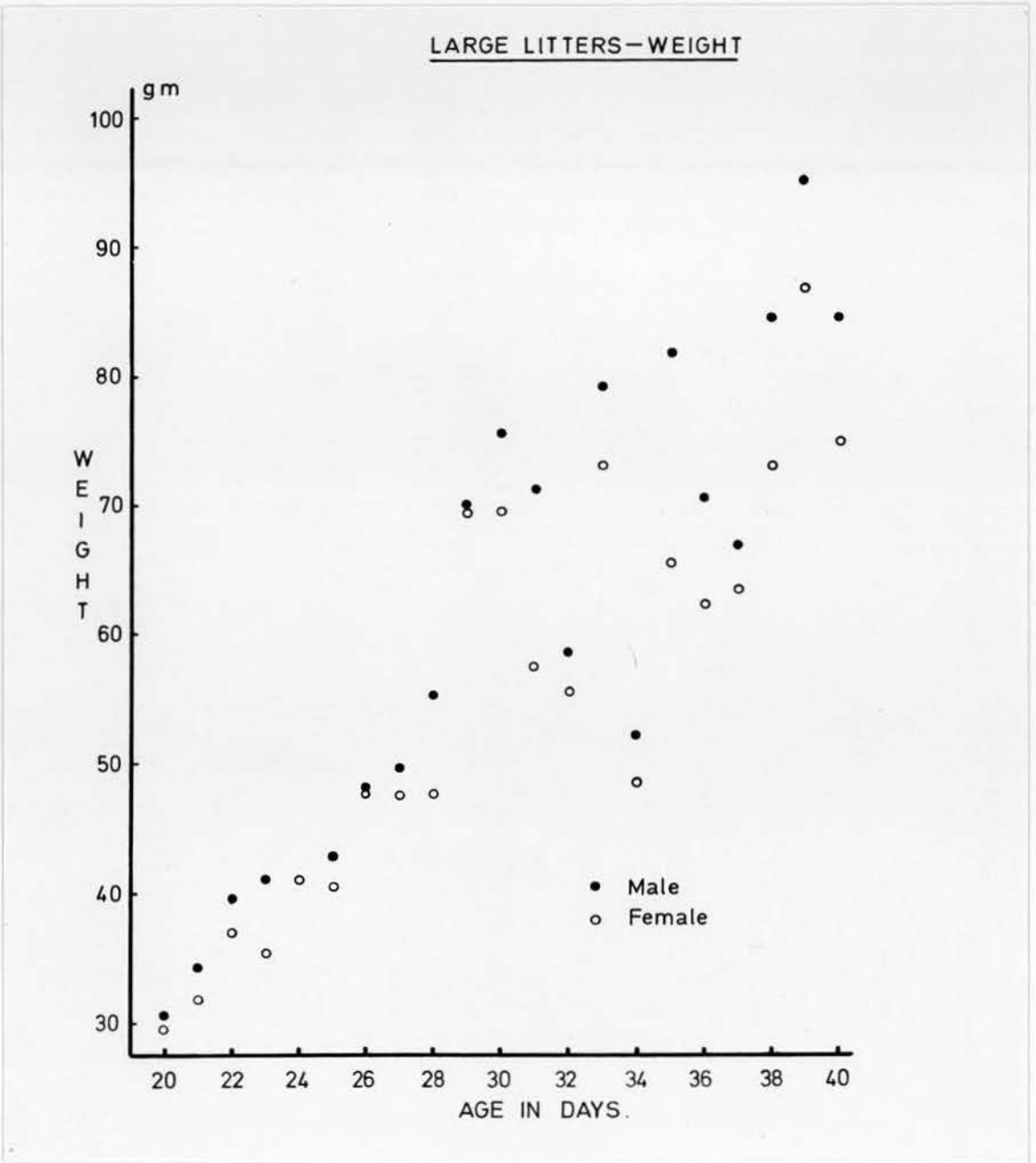

LARGE LITTERS—WEIGHT
gm
100
90
80
70
60
50
40
30
WEIGHT
20
22
24
26
28
30
32
34
36
38
40
AGE IN DAYS.
Male
Female

Figure 33 Distribution graph plotted on the means of the small litter lengths of both males and females covering a period of 20 to 40 days.

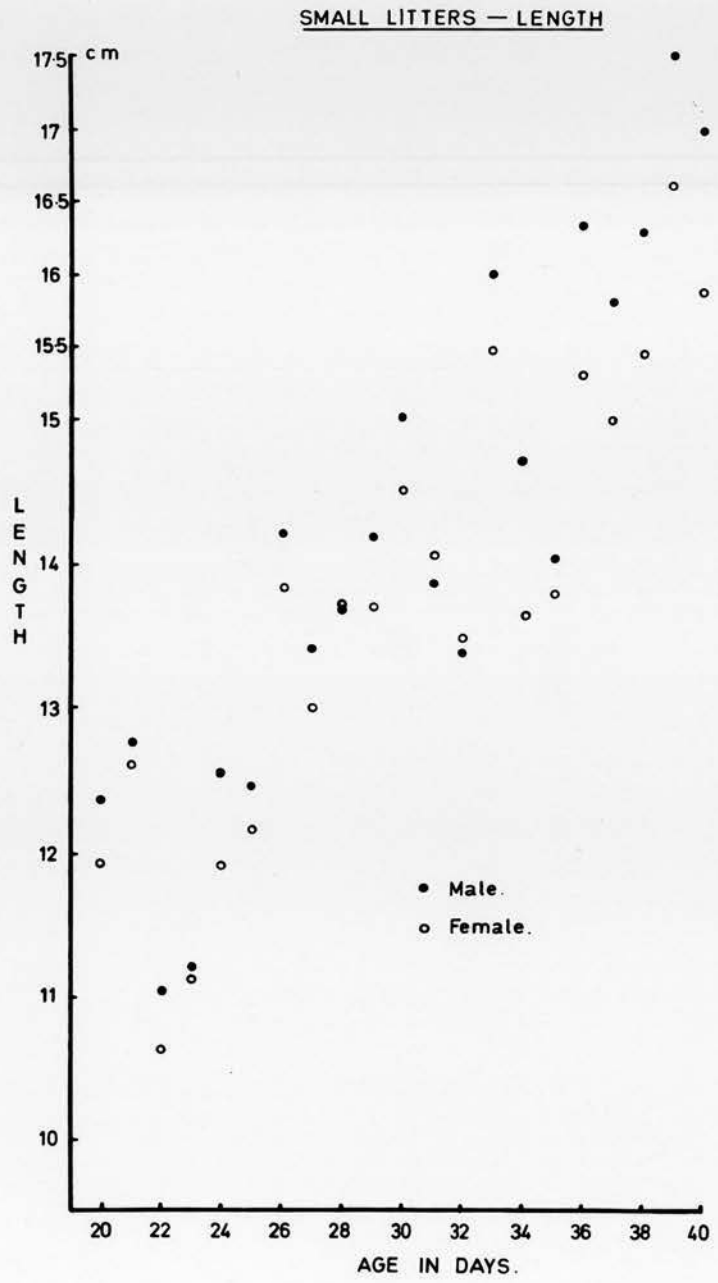
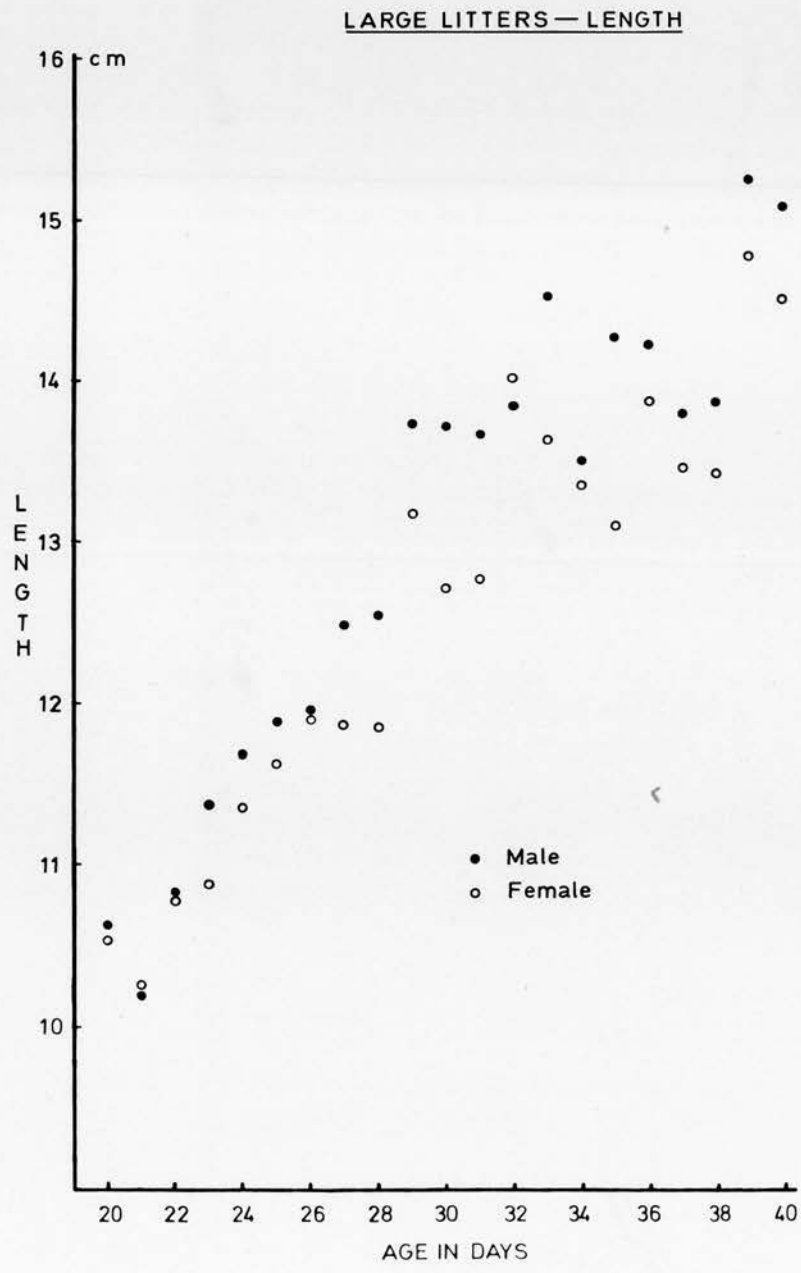

SMALL LITTERS — LENGTH
cm
17·5
17
16·5
16
15·5
15
14
13
12
11
10
LENGTH
20
22
24
26
28
30
32
34
36
38
40
AGE IN DAYS.
Male.
Female.

Figure 34 Distribution graph plotted on the means of the large litter lengths of both males and females covering a period of 20 to 40 days. Compare with Figure 33 and note that the large litter length reflects retardation.


LARGE LITTERS — LENGTH
16
cm
15
14
13
12
11
10
LENGTH
Male
Female
20
22
24
26
28
30
32
34
36
38
40
AGE IN DAYS

the males do enjoy a slight weight advantage on these mean calculations (fig. 31) but it is difficult to construe this as due to a sex difference. From day 30 to day 40 the weight differences increase and this is made more evident when the male mean at day 30 shows a 5 g difference compared to the female mean. Following day 30 there is a certain amount of fluctuation where males and females can draw quite close but from day 35 onward the sex difference by weight is well marked.

In the large litters, where weight is much less on day 21 (approximately 20g) compared to the same period in the small litters, there appears no significant mean weight differences which can be ascribed to a sexual division Generally, the males are a fraction heavier (fig. 32) and this state remains until day 30. Beyond day 30, although there is a slight fluctuation, the male attains a definite greater weight than the female.

Inspection of the division of the sexes by length shows that in the small litters the majority of the males are longer than the females with the more marked differences appearing beyond day 33. Figure 34 shows the plot for the large litters which reveals that although there are differences, they are small ones and that even by days 30 to 40 they remain small compared to those of the small litters. Obviously the growth process in length is only recovering lost ground very slowly.

Deficits in weight and length do not appear to have altered the basic differences which characterise the sexes, only that because the results are not so well defined as in small litters it is difficult to separate sex differences in some of the results from litter variance under retarding conditions. The characteristic sex weight-length pattern has been blurred in the large litters simply because the parameters used to elucidate it have undergone a general alteration.

Weight of the small litters at day 21 is approximately 20 grams heavier than

that of the large litters - a result of the preweaning conditions. From day 33 the small litters are found to be moving over the 100g mark, after which the individual means of the litters fluctuate over a range of some 50g. In the large litters, however, there is a gradual increase in weight up to around the 30 day mark followed by an upsurge of weight. This upsurge of weight moves above the 60g mark but only once (day 39) does it reach the 95g level, the remaining litter means staying below the 88g level. The 20 days of unstinted food supply is not long enough to allow the large litters to both increase the growth and weight at the same time relative to the small litters.

Over the period day 20 to 25, the head-body length of the small litters (fig. 33) shows considerable litter variance whereas the length of large litters (fig. 34) over the same period although slightly behind, shows a steady growth pattern. From 25 days to 40 days, the small litter lengths show a steady increase with fluctuations of approximately 4 cms. In comparison, the large litter lengths remain at a slow growth rate until around day 30 after which an increase is found, also with some degree of fluctuation, reaching approximately 2.75 cms by day 40. As in the situation with weight the catch-up period for length has not been sufficient although compared to weight the relative difference between the large and small litters was less.

4.8 Patterns of Body Growth - Postweaning Discussion

Growth curves of rodents generally show evidence of segments or cycles and these in turn reflect the decrease and increase of the growth rates. So far, the postnatal development has been divided into two stages - a preweaning and a postweaning. The watershed between these two has been arbitrarily placed at 20 days following birth since this particular time, or approximately this time, is when many rat litters are observed as having been weaned. In Section 4.3 the first 20 days were found to be triphasic in character with true biological weaning

beginning some time near the 11th day and being completed before day 20 had been reached. The concept of triphasic growth is too new to allow a change of the preweaning-postweaning watershed to be used in a general discussion so that, although we are aware of the triphasic phenomenon the term "preweaning" period is reserved to mean birth to 20 days.

The next term which now gives rise to a problem is that of "postweaning" in the sense that the term relates to the "weaning" of the animal. Where, therefore, does one end the postweaning stage? Let us presume that it is related to the growth and food supply of the developing rat then development must cease in its more active form at some point. In relation to this, postnatal growth as a whole can be classified simply as pre- and post pubertal phases which means shifting the watershed summit further along the age parameter. A break in the growth curve has been generally accepted as coinciding with sexual maturity, and this in fact is a manifestation of many physiological changes. In the rat, as in any other mammal, the transition from one state to another means that there are alterations in the basal and resting metabolism as linked with body size, the involution of the thymus, relative growth changes and many others linked with the shift or hormonal balance. In the albino laboratory rat these changes are regarded as taking place when the body weight is approximately 100 grams (BERTALANFFY, 1960).

On the basis of the above discussion, it could be claimed that the postweaning phase of development could be placed within the prepubertal stage by coinciding its termination at the same time, i.e. when the rat reached 100 grams. On this premise, the small litters could be accepted as having "passed" the 100 gram barrier at day 33, and this is reasonable when it is noted from Figure 31 that the sexual dimorphism of the rats also becomes clearly defined. On the other hand, Figure 32 defeats the whole purpose of this when it is noted that the large

litters do not rise above the 100 gram mark. Obviously the postweaning phase as defined by the pubertal changes can only apply to small litters. Because of the difficulties outlined, for purposes of this study the term "postweaning" applies to the period of time encompassed between day 20 and day 40 (the end of the experiment).

Nutritional effects are known to have a direct link with the attaining of puberty. This attainment is a function of the size of the animal as well as its age, so that if there are changes in the growth rate, as in the large litters, there will be a change in attainment of any definite size. It has been suggested that below a certain minimal size an individual rat cannot reach puberty independent of its nutritional status. JOUBERT (1963) postulated in a review of puberty of various animals that they could not reach puberty until a certain degree of physical development had been reached typical of their kind. Support of this has been given by RUSSELL (1948) who found that a reduction in the plane of nutrition delayed puberty. Experimentally he found that rats fed on 1/3 to 1/4 of their normal calorie intake failed to reach sexual maturity after 12 weeks of weaning whereas the controls did. Various Vitamin deficiencies have been mentioned by MOUSTGAARD (1959) who also pointed out that gross underfeeding reduces the gonadotrophic potential of the adult pituitary. Thus disturbances stemming from this kind of dietary regime appear to be due to a reduced pituitary production rather than a fall in responsiveness of the gonads. This principle can be applied to young developing rats where undernutrition as found in large litters affects the growth rate and may also affect the output of the pituitary-based growth hormones and hence have a two-pronged effect on the attainment of size necessary for puberty.

The alterations of the time required to achieve puberty can not be answered in the present work since the period of time studied falls rather short of the

generally accepted time, furthermore the weight of 100 grams suggested by BERTALANFFY (1960) is not valid for the large litters.

A generally accepted biological fact is that following a temporary cessation of growth and also from different sizes at the beginning, many animals reach the same species-characteristic final size. Examples of a temporary suspension of growth can be observed if a diet is lacking both quantity and quality. This aspect was investigated experimentally using rodents and other animals and it was found that the growth was slowed down to a point where there was no relative increase of body weight although it appeared to be maintained at a reasonable level. When normal diet was resumed the animals eventually reached the normal final projected weight (KOPEC, 1938; CLARKE and SMITH, 1938; JACKSON, 1939; BERTALANFFY, 1960). With litters of varying size the weight of the animal at birth is normally low when it is a member of a large litter whereas in a small litter the weight is greater. Since there are many factors which could influence the size of the animal at birth, this kind of difference must be taken as a generalisation although KOPEC (1932) showed that the same final weight is eventually attained by both the large and small animals at birth. This type of recovery of growth only occurs if the period of malnutrition has not inflicted lasting damage on the developing tissues, and this was supported by the work of HAZEL, BAKER and REINMILLER (1943) and KNAPP and CLARKE (1947, 1950). Basically, if there is severe undernutrition the extent to which the animal recovers depends upon the age of onset and the duration of the nutritional deficits (SCHULTZE, 1955; WIDDOWSON and McCANCE, 1963; WINICK and NOBLE, 1966; BROWN and GUTHRIE, 1968). When the animals are unable to regain their deficits during the postweaning period this backward development is regarded by WIDDOWSON and KENNEDY (1962) as being related to the increased hereditary control over gain in weight and size after weaning and to the rate of multiplication which decreased with time.

Within the present context, the observations show that the large litters had undergone a level of retardation which has been unable to regain ground inside the period of time. Since the undernutrition took place during the first 20 days it is evident that the following 20 days were insufficient to allow the transfer and food assimilation to take full advantage. Two possibilities emerge related to this retardation, firstly that the growth potential has been damaged in some way and, secondly that the growth potential no longer operates at the same speed, hence irrespective of energy supplies only a limited amount of growth is possible in the allotted time. In any case, the normal maintenance level of the body - remembering that there is a fall of nearly 50% of weight - would require correction before lost growth be undertaken.

WIDDOWSON, DICKERSON and McCANCE (1960) have suggested that the consequences of altering growth may differ depending on the state of development reached when the change is made. This can be due to a change in the rate of growth that does not conform with the change in the rate of maturation.

Under conditions where growth was retarded using experimental animals, it was noted that the progress of maturation was not retarded to the same level (ACHESON and MacINTYRE, 1958; DICKERSON and WIDDOWSON, 1960). This finding was regarded as corresponding to observations in humans who had undergone severe disturbances arising from illness or famine (ACHESON and HEWITT, 1954; JEFFREYS, 1969). Growth restriction in male rats produced permanent dwarfing unless the restriction was imposed after 60 days (WIDDOWSON and McCANCE, 1963). Investigations of animals which had made a partial recovery, the physical and chemical composition was found to have a less mature form than normal animals. The important point which arose from this work (DICKERSON and WIDDOWSON, 1960) was that the changes did not affect all parts uniformly so that the presence of "priority growth" is indicated based on the fact that in earlier developing parts the effect was less.

Although the present work only covers the first 40 days of life, the differences in the weight and length between the small and large litters remain outstanding and the question then arises on whether the deficit would be erased in time. By simple extrapolation some of the litters might well reach their final species characteristic size - and those that do probably owe that achievement to the original high level of lactation of the mother, or that the litter was slightly small and thus suited the maternal capacity. When WIDDOWSON and McCANCE (1960) decreased the litters to 3 and 18 young, very large differences between the two types of litter emerged and at weaning the weights of the large litters were nearly 50% under normal.

In recent work SCHEMMEL, MICKELSEN and FISHER (1973) commented that the observations of WIDDOWSON and McCANCE (1960) might be interpreted as indicating that preweaning body weight gain influences the subsequent somatic development. This was based on the fact that following weaning of the larger litters the lighter rats of these litters never achieved the same weight as the small litter rats. The differences in body weight were present in the adult state in spite of the lift of food restriction after weaning. The implication from these observations is that the larger, i.e. the greater, the body weight gain prior to weaning the larger the ultimate size of the animal.

Obviously the principle of equifinality cannot be followed when retardation of this level is reached. Already mentioned are the variables introduced by the maternal capacity as well as litter-size, and within the large litters of the present work there are fluctuations of numbers ranging from 13 to 18 (Figs. 28, 30). The mean trend of growth of both length and weight are clearly indicated but the range of weight and length within one of these large litters fluctuates probably as much as the individual mother's lactational capacity. Thus within one large litter there are very light rats and heavier rats - the heavier rats

are not comparable with rats from the small litter. If size prior to weaning is a forecast of the final size in the adult stage, then the heavier rats of the large litters will probably follow BERTALANFFY's "Principle of equifinality" whereas the lighter rats will remain slightly stunted. In simpler terms, the lighter rats of the larger litters have sustained damage or damages to their growth potential at a crucial point of development.

The observations of the present work can only be interpreted within the time limits set by the experiments and therefore it is obvious that the "recovery" has not occurred by day 40 and the possibilities of such a goal being reached lie within the speculative field.

In a wider approach, the effects of the environmental and genetic variation of animal growth and size have been investigated by a number of workers such as MILLS (1945), CAWLEY, McKEOWN and RECORD (1954), ACHESON (1960), HAMMOND (1961), HARRISON (1963), CHEVILLARD, PORTET and CADOT (1963) and PORTER and FESTING (1969). They did not, however, dwell much on the effects on the morphology. The importance of the morphological aspects during development lies in the fact that they allow a means of analysing the extent to which the correlations between the patterns of maturation and growth can be altered and, equally important, the extent to which the various processes such as the growth potential acting on or with various regions or parameters can be shown to be independent. The maturation process has a fixed chronological position in relation to the general growth of the animal although it will have a range of variance within the framework of normality. Investigations in relation to the body weights of rodents with morphological maturation in mind have been undertaken by numerous workers (ENGLE and ROSASCO, 1927; PARKES, 1929; OUTHOUSE and MENDEL, 1933; KENNEDY, 1957; BIGGERS et al, 1958; WIDDOWSON and McCANCE, 1960; KNUDSEN, 1962; BARNETT and BURN, 1967; GALL and KYLE, 1968; GARRARD, HARRISON and WEINER, 1974) and they

found that the results varied considerably according to the conditions under which the animals were reared and to the particular morphological aspects observed.

The use of nutrition variation as the environmental change has been employed in the present work and has also been used by PARKES (1929), YOON (1955), KENNEDY (1957), and WIDDOWSON and McCANCE (1960), and all showed that there were changes in body weight. Maturation - in whatever form it may be observed by - seems by general principles to have its age of occurrence to be dependent on the body weight. Thus, if young rats have lost approximately 50% of their weight during membership of a large litter then their growth is retarded and hence their maturation. The criteria of what constitutes maturation has reservations since not all the normal morphological features respond to the changes. GARRARD, HARRISON and WEINER (1974) have shown that the ages of the eruption of mandibular incisors, the appearance of nipples and, to a lesser extent, the opening of the vagina could all be related to the body weight of the animal hence general growth patterns. On the other hand, they observed that the opening of the eye and the "unpinning" of the ears continued to occur irrespective of the animal's growth state.

In short, although the animal exhibits a general growth pattern some of the underlying processes have a different growth control and can be independently affected by the genetic endowment and the environmental factors. It must be pointed out that although growth has been described in terms of weight and length and correlated to morphological events and age, they are at best clumsy parameters of growth changes and the underlying factors are of a much more complex nature.

4.9 Patterns of Body Growth - Postweaning Summary

The postweaning development of small and large litters, based on the cross-

fostering technique at birth, was examined at 24-hourly intervals over a period of 20 days, i.e. day 20 to day 40. The characteristic changes of both weight and length were examined by means of a distribution chart and the sexes separated. The most obvious change, apart from the much slower growth of the large litters compared to the small litters, was the variance within each litter type. It is suggested that this variance stems from the restrictions of the maternal environment being lifted and the full play of the genetic endowment is being allowed to express itself. Sexual dimorphism was not found to be significant before day 30, thereafter the difference by weight and length became more evident. From day 33 the small litter weights pass the 100g mark but the large litters do not reach this level by the end of the experimental period (day 40). It is evident that the catch-up period of some 20 days is not the only factor which may be insufficient, there is also the problem of damage to the growth mechanism and the timing of the growth potential. Obviously the growth potential during the preweaning period is extremely susceptible to adverse food conditions. Within every large litter there exists a considerable range of individual weights attained and it appears that the heavier individuals, by size alone, are better suited to reach their characteristic species-size (principle of equifinality) than their smaller lighter litter-mates. The conditions induced on the maternal capacity of large litters ranging from 13 to 18 individuals per litter during the preweaning period have, without doubt, a marked and apparently lasting influence on the growth potential of the rats over the postweaning period even in the presence of unrestricted food supplies.

The data supplied does not give sufficient evidence from which to extrapolate the recovery or non-recovery of the growth. Apart from the amount of variance already operating in the field, the length of period of observation would have to be extended considerably beyond the 40 day limits to allow confirmation or refutation of the principle of equifinality.

4.10 TABLES OF BODY ANALYSIS

GROWTH SURVEY

CONTROL

FIRST SAMPLE

BIRTH - 20 DAYS

GROWTH SURVEY — BODY

Litter Ref. A/EL 29 — Age: BIRTH

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	6.0	5.0	2.751	0.2400	0.047
	Range	5.7 - 6.2	5.0 - 5.1			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	6.06	5.0	2.722	0.2424	0.485
	Range	5.9 - 6.2	5.0			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	6.03	5.0	2.737	0.2393	0.047
	Range	5.7 - 6.2	5.0 - 5.1			
	S.E. of Mean	0.0794	0.0173			
	Coeff. of Variation	3.1%	0.9%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 53 — Age: 1DAY

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	6.93	5.54	2.902	0.2258	0.0410
	Range	6.7 - 7.1	5.5 - 5.6			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	7.03	5.6	2.906	0.2242	0.0389
	Range	6.8 - 7.3	5.5 - 5.8			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	6.98	5.5	2.904	0.2258	0.0399
	Range	6.7 - 7.3	5.5 - 5.8			
	S.E. of Mean	0.0878	0.0519			
	Coeff. of Variation	3.1%	2.3%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 32 — Age: 2 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	8.13	5.76	2.867	0.2450	0.0442
	Range	7.9 - 8.5	5.6 - 5.9			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	8.16	6.03	2.997	0.2244	0.0372
	Range	7.5 - 8.6	5.9 - 6.2			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	8.15	5.9	2.932	0.2345	0.0407
	Range	7.5 - 8.6	5.6 - 6.2			
	S.E. of Mean	0.1622	0.0489			
	Coeff. of Variation	4.0%	2.9%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 57 — Age: 2 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	7.3	5.7	2.940	0.2247	0.0394
	Range	6.8 - 8.1	5.6 - 5.8			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	7.7	5.8	2.929	0.2289	0.0399
	Range	7.5 - 8.0	5.7 - 5.9			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	7.5	5.7	2.935	0.2278	0.0396
	Range	6.8 - 8.1	5.6 - 5.9			
	S.E. of Mean	0.2184	0.0412			
	Coeff. of Variation	7.1%	1.74%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 33 — Age: 3 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	8.96	6.06	2.921	0.2440	0.0402
	Range	8.3 - 9.4	6.0 - 6.1			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	9.03	6.03	2.897	0.2483	0.0412
	Range	8.7 - 9.5	6.0 - 6.1			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	9.0	6.0	2.909	0.2461	0.0407
	Range	8.3 - 9.5	6.0 - 6.1			
	S.E. of Mean	0.1863	0.0436			
	Coeff. of Variation	5.1%	1.7%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 56 — Age: 3 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	9.1	6.1	2.936	0.2446	0.0395
	Range	8.7 - 9.4	5.9 - 6.3			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	8.6	5.9	2.891	0.2471	0.0415
	Range	8.4 - 8.9	5.7 - 6.1			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	8.8	6.0	2.914	0.2438	0.0405
	Range	8.4 - 9.4	5.7 - 6.3			
	S.E. of Mean	0.1540	0.0877			
	Coeff. of Variation	4.2%	3.56%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 34 — Age: 4 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	9.83	6.1	2.847	0.2642	0.0434
	Range	9.6 - 10.1	6.0 - 6.2			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	9.83	6.3	2.893	0.2477	0.0394
	Range	9.6 - 10.0	6.2 - 6.4			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	9.83	6.2	2.870	0.2561	0.0414
	Range	9.6 - 10.1	6.0 - 6.4			
	S.E. of Mean	0.0854	0.0877			
	Coeff. of Variation	2.1%	4.7%			

GROWTH SURVEY — BODY

Litter Ref. A/2C/47 — Age: 4 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	10.2	6.7	3.099	0.2272	0.0336
	Range	9.7 - 11.2	6.6 - 6.9			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	10.1	6.6	3.065	0.2319	0.0347
	Range	9.8 - 10.6	6.5 - 6.7			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	10.1	6.6	3.082	0.2283	0.0341
	Range	9.7 - 11.2	6.5 - 6.9			
	S.E. of Mean	0.2381	0.0548			
	Coeff. of Variation	5.7%	2.01%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 48 — Age: 5 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	10.6	6.5	2.952	0.2509	0.0389
	Range	10.2 - 11.0	6.4 - 6.7			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	10.4	6.4	2.957	0.2539	0.0389
	Range	9.9 - 10.8	6.3 - 6.6			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	10.5	6.4	2.955	0.2517	0.0389
	Range	9.9 - 11.0	6.3 - 6.7			
	S.E. of Mean	0.1507	0.0755			
	Coeff. of Variation	3.5%	3.9%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 54 — Age: 5 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	10.9	6.8	3.063	0.2357	0.0349
	Range	10.6 - 11.2	6.6 - 7.0			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	11.1	6.8	3.064	0.2401	0.0348
	Range	10.2 - 11.6	6.6 - 7.1			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	11.0	6.8	3.063	0.2372	0.0349
	Range	10.2 - 11.6	6.6 - 7.1			
	S.E. of Mean	0.2025	0.0837			
	Coeff. of Variation	4.5%	3.0%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 4 — Age: 6 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	13.63	6.27	2.692	0.3482	0.0513
	Range	13.3 - 14.0	6.3 - 6.65			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	11.1	6.66	2.991	0.2503	0.0392
	Range	10.6 - 11.5	6.1 - 7.4			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	12.36	6.5	2.842	0.2927	0.0453
	Range	10.6 - 14.0	6.1 - 7.4			
	S.E. of Mean	0.5857	0.1141			
	Coeff. of Variation	11.6%	5.6%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 45 — Age: 6 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	13.9	6.9	2.875	0.2920	0.0424
	Range	11.9 - 15.4	6.7 - 7.2			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	13.5	6.8	2.869	0.2920	0.0424
	Range	12.4 - 14.7	6.5 - 7.2			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	13.7	6.8	2.872	0.2905	0.0424
	Range	11.9 - 15.4	6.5 - 7.2			
	S.E. of Mean	0.5597	0.1140			
	Coeff. of Variation	10.0%	4.07%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 5 — Age: 7 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	14.3	6.9	2.853	0.3004	0.0431
	Range	13.9 - 14.3	6.7 - 7.0			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	13.3	6.9	2.910	0.2794	0.0406
	Range	12.9 - 13.8	6.8 - 7.0			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	13.7	6.9	2.881	0.2884	0.0419
	Range	12.9 - 14.3	6.7 - 7.0			
	S.E. of Mean	0.2122	0.0520			
	Coeff. of Variation	3.8%	1.8%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 6 — Age: 8 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	17.66	7.58	2.905	0.3074	0.0402
	Range	17.2 - 18.5	7.55 - 7.6			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	16.73	7.4	2.892	0.3055	0.0413
	Range	16.3 - 17.3	7.3 - 7.5			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	17.19	7.4	2.899	0.3058	0.0408
	Range	16.3 - 18.5	7.3 - 7.6			
	S.E. of Mean	0.3099	0.0520			
	Coeff. of Variation	4.4%	1.7%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 22 — Age: 9 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	21.1	7.9	2.858	0.3381	0.0428
	Range	20.2 - 21.9	7.7 - 8.0			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	20.4	7.93	2.903	0.3244	0.0409
	Range	20.1 - 20.8	7.8 - 8.1			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	20.7	7.9	2.881	0.3308	0.0419
	Range	20.1 - 21.9	7.7 - 8.1			
	S.E. of Mean	0.2859	0.0609			
	Coeff. of Variation	3.4%	1.9%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 7 — Age: 10 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	21.45	8.05	2.897	0.3310	0.0404
	Range	21.3 - 21.6	8.0 - 8.1			
Male	S.E. of Mean					
	Coeff. of Variation					
	Mean	20.6	8.0	2.918	0.3219	0.0408
	Range	20.3 - 21.0	7.8 - 8.2			
Female	S.E. of Mean					
	Coeff. of Variation					
	Mean	20.8	8.0	2.911	0.3253	0.0406
	Range	20.3 - 21.6	7.9 - 8.2			
Combination	S.E. of Mean	0.2090	0.0510			
	Coeff. of Variation	2.5%	2.2%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 43 — Age: 10 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	22.3	8.1	2.939	0.3399	0.0410
	Range	15.4 - 27.7	8.1 - 8.2			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	22.0	8.2	2.964	0.3272	0.0396
	Range	14.6 - 26.2	8.0 - 8.4			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	22.2	8.1	2.951	0.3304	0.0403
	Range	14.6 - 27.7	8.0 - 8.4			
	S.E. of Mean	2.3337	0.0548			
	Coeff. of Variation	25.7%	1.64%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 12 — Age: 11 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	24.4	8.4	2.907	0.3458	0.0408
	Range	24.0 - 24.8	8.2 - 8.6			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	23.4	8.36	2.923	0.3348	0.0400
	Range	22.7 - 24.2	8.2 - 8.6			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	23.9	8.4	2.915	0.3390	0.0404
	Range	22.7 - 24.8	8.2 - 8.6			
	S.E. of Mean	0.3082	0.0775			
	Coeff. of Variation	3.2%	2.3%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 38 — Age: 12 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	30.93	9.03	3.878	0.3793	0.0420
	Range	28.5 - 32.3	8.7 - 9.2			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	29.96	9.16	2.952	0.3571	0.0389
	Range	28.8 - 32.1	8.9 - 9.3			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	30.4	9.1	2.915	0.3677	0.0405
	Range	28.5 - 32.3	8.7 - 9.3			
	S.E. of Mean	0.7565	0.1006			
	Coeff. of Variation	6.1%	2.7%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 13 — Age: 13 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	31.0	9.33	2.970	0.3561	0.0382
	Range	30.5 - 31.3	9.2 - 9.5			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	31.5	9.26	2.934	0.3674	0.0397
	Range	30.2 - 32.2	9.0 - 9.4			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	31.2	9.3	2.952	0.3617	0.0390
	Range	30.2 - 32.2	9.0 - 9.5			
	S.E. of Mean	0.3071	0.0448			
	Coeff. of Variation	2.4%	1.2%			

Litter Ref. A/EL 14 Age: 14 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	31.2	9.24	2.933	0.3654	0.0397
	Range	29.7 - 32.1	9.0 - 9.5			
Male	S.E. of Mean					
	Coeff. of Variation					
	Mean	31.56	9.33	2.952	0.3626	0.0388
	Range	31.1 - 32.0	9.2 - 9.5			
Female	S.E. of Mean					
	Coeff. of Variation					
	Mean	31.3	9.28	2.942	0.3644	0.0393
	Range	29.7 - 32.1	9.0 - 9.5			
Combination	S.E. of Mean	0.3665	0.1591			
	Coeff. of Variation	2.6%	5.6%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 60 — Age: 14 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	32.3	10.2	3.222	0.3105	0.0299
	Range	30.6 - 35.0	10.2 - 10.3			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	33.8	10.3	3.194	0.3186	0.0307
	Range	33.6 - 34.1	10.2 - 10.4			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	33.1	10.2	3.208	0.3120	0.0303
	Range	30.6 - 35.0	10.2 - 10.4			
	S.E. of Mean	0.6871	0.0361			
	Coeff. of Variation	5.1%	0.87%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 28 — Age: 15 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	33.63	9.73	3.014	0.3552	0.0366
	Range	32.7 - 34.5	9.5 - 10.0			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	35.36	9.86	3.006	0.3637	0.0368
	Range	33.6 - 36.5	9.6 - 10.0			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	34.5	9.8	3.016	0.3594	0.0367
	Range	32.7 - 36.5	9.5 - 10.0			
	S.E. of Mean	0.5997	0.0933			
	Coeff. of Variation	4.3%	2.3%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 24 — Age: 16 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	37.1	16.26	3.078	0.1403	0.0343
	Range	36.9 - 37.4	16.2 - 16.3			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	38.16	10.53	3.128	0.3422	0.0327
	Range	38.0 - 38.3	10.5 - 10.6			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	37.63	10.4	3.103	0.3479	0.0335
	Range	36.9 - 38.3	10.2 - 16.6			
	S.E. of Mean	0.2510	0.0633			
	Coeff. of Variation	1.6%	1.5%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 23 — Age: 17 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	40.93	11.3	3.230	0.3205	0.0297
	Range	39.9 - 42.4	10.9 - 11.5			
Male	S.E. of Mean					
	Coeff. of Variation					
	Mean	41.06	11.06	3.205	0.3357	0.0306
	Range	38.2 - 43.0	10.2 - 11.6			
Female	S.E. of Mean					
	Coeff. of Variation					
	Mean	40.99	11.1	3.217	0.3336	0.0302
	Range	38.2 - 43.0	10.2 - 11.6			
Combination	S.E. of Mean	0.7362	0.2433			
	Coeff. of Variation	4.4%	8.1%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 61 — Age: 17 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	40.1	9.7	2.842	0.4262	0.0436
	Range	39.4 - 41.1	9.6 - 9.8			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	38.5	9.5	2.832	0.4266	0.0440
	Range	37.1 - 40.2	9.4 - 9.8			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	39.3	9.6	2.837	0.4225	0.0438
	Range	37.1 - 41.1	9.4 - 9.8			
	S.E. of Mean	0.5941	0.0707			
	Coeff. of Variation	3.7%	1.79%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 16 — Age: 18 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	40.3	10.56	3.081	0.3614	0.0344
	Range	39.5 - 41.1	10.1 - 11.1			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	40.0	10.83	3.167	0.3410	0.0315
	Range	39.3 - 41.3	10.5 - 11.1			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	40.15	10.69	3.124	0.3518	0.0330
	Range	39.3 - 41.3	10.1 - 11.1			
	S.E. of Mean	0.3629	0.0959			
	Coeff. of Variation	2.2%	3.1%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 50 — Age: 18 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	41.4	11.0	3.201	0.3397	0.0306
	Range	38.8 - 44.9	10.9 - 11.3			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	39.3	10.9	3.219	0.3308	0.0302
	Range	35.5 - 43.2	10.8 - 11.0			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	40.3	11.0	3.210	0.3340	0.0304
	Range	35.5 - 44.9	10.8 - 11.3			
	S.E. of Mean	1.3622	0.0686			
	Coeff. of Variation	8.3%	1.52%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 18 — Age: 19 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	44.16	10.56	2.988	0.3960	0.0375
	Range	43.1 - 45.2	10.4 - 10.9			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	41.96	10.3	2.936	0.3955	0.0395
	Range	38.6 - 44.9	10.0 - 10.9			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	43.06	10.3	2.962	0.3994	0.0385
	Range	38.6 - 45.2	10.0 - 10.9			
	S.E. of Mean	0.9931	0.1573			
	Coeff. of Variation	5.7%	5.1%			

GROWTH SURVEY — BODY

Litter Ref. A/2C 52 — Age: 19 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	42.5	10.9	3.142	0.3577	0.0323
	Range	39.0 - 44.5	10.4 - 11.4			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	45.6	11.2	3.143	0.3635	0.0323
	Range	42.8 - 47.9	10.6 - 11.7			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	44.0	11.0	3.142	0.3577	0.0323
	Range	39.0 - 47.9	10.4 - 11.7			
	S.E. of Mean	1.2478	0.2066			
	Coeff. of Variation	6.9%	4.56%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 31 — Age: 20 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	47.4	11.01	3.058	0.3910	0.0350
	Range	46.8 - 48.2	10.9 - 11.2			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	47.4	10.63	2.036	0.4195	0.0396
	Range	46.9 - 48.0	10.3 - 10.9			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	47.4	10.8	2.997	0.4039	0.0373
	Range	46.8 - 48.2	10.3 - 11.2			
	S.E. of Mean	0.12339	0.2088			
	Coeff. of Variation	1.2%	6.8%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 21 — Age: 20 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	45.3	10.6	2.982	0.4032	0.0379
	Range	39.7 - 48.9	9.7 - 11.1			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	36.7	10.2	3.105	0.3527	0.0335
	Range	23.6 - 44.6	8.8 - 11.1			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	40.97	10.4	3.044	0.3714	0.0357
	Range	23.6 - 48.9	8.8 - 11.1			
	S.E. of Mean	3.7367	0.3946			
	Coeff. of Variation	22.3%	9.27%			

GROWTH SURVEY

CONTROL

SECOND SAMPLE

1 - 40 DAYS

GROWTH SURVEY — BODY

Litter Ref. CL/92 and CL/90 — Age: 1 DAY

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	6.00	5.41	2.982	0.2050	0.0379
	Range	5.4 - 6.8	5.2 - 5.8			
Male	S.E. of Mean	0.1761	0.0735			
	Coeff. of Variation	7.76%	3.61%			
	Mean	5.54	5.42	3.064	0.1886	0.0348
	Range	5.4 - 5.8	5.2 - 5.7			
Female	S.E. of Mean	0.0748	0.0860			
	Coeff. of Variation	3.02%	3.55%			
	Mean	5.81	5.42	3.016	0.1978	0.0365
	Range	5.4 - 6.8	5.2 - 5.8			
Combination	S.E. of Mean	0.1241	0.0539			
	Coeff. of Variation	7.39%	3.41%			

GROWTH SURVEY — BODY

Litter Ref. CL/93 and CL/71 — Age: 2 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	6.94	5.73	3.004	0.2114	0.0369
	Range	6.5 - 7.26	5.3 - 6.2			
Male	S.E. of Mean	0.1526	0.1304			
	Coeff. of Variation	5.82%	6.02%			
	Mean	6.54	5.86	3.137	0.1905	0.0325
	Range	5.6 - 7.4	5.6 - 6.2			
Female	S.E. of Mean	0.3341	0.1208			
	Coeff. of Variation	11.42%	4.61%			
	Mean	6.78	5.78	3.059	0.2029	0.0351
	Range	5.6 - 7.6	5.3 - 6.2			
Combination	S.E. of Mean	0.1670	0.0894			
	Coeff. of Variation	8.53%	5.36%			

GROWTH SURVEY — BODY

Litter Ref. CL/72 and CL/73 — Age: 3 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	8.23	6.03	2.994	0.2267	0.0375
	Range	6.4 - 10.5	5.6 - 6.9			
	S.E. of Mean	0.5233	0.1543			
	Coeff. of Variation	17.98%	7.24%			
Female	Mean	6.73	5.70	3.018	0.2071	0.0363
	Range	5.9 - 7.7	5.4 - 6.3			
	S.E. of Mean	0.4553	0.2121			
	Coeff. of Variation	13.53%	7.44%			
Combination	Mean	7.73	5.92	3.002	0.2206	0.0373
	Range	5.9 - 10.5	5.4 - 6.9			
	S.E. of Mean	0.4249	0.1277			
	Coeff. of Variation	19.04%	7.48%			

GROWTH SURVEY — BODY

Litter Ref. CL/73 and CL/121 — Age: 4 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	11.56	6.96	3.087	0.2386	0.0343
	Range	9.3 - 13.7	6.6 - 7.2			
	S.E. of Mean	0.5790	0.0721			
	Coeff. of Variation	13.25%	2.73%			
Female	Mean	11.22	6.88	3.082	0.2370	0.0345
	Range	9.3 - 12.7	6.6 - 7.2			
	S.E. of Mean	0.6733	0.1200			
	Coeff. of Variation	13.42%	3.90%			
Combination	Mean	11.42	6.93	3.085	0.2378	0.0343
	Range	9.3 - 13.7	6.6 - 7.2			
	S.E. of Mean	0.4218	0.0632			
	Coeff. of Variation	12.79%	3.14%			

GROWTH SURVEY — BODY

Litter Ref. CL/76 and CL/120 — Age: 5 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	13.09	7.22	2.067	0.2507	0.0348
	Range	11.5 - 16.0	6.7 - 8.0			
	S.E. of Mean	0.4414	0.1517			
	Coeff. of Variation	10.12%	6.30%			
Female	Mean	12.77	7.27	3.113	0.2416	0.0332
	Range	12.3 - 13.3	7.2 - 7.4			
	S.E. of Mean	0.2907	0.0671			
	Coeff. of Variation	3.94%	1.59%			
Combination	Mean	13.01	7.23	3.078	0.2489	0.0344
	Range	11.5 - 16.0	6.7 - 8.0			
	S.E. of Mean	0.3345	0.1131			
	Coeff. of Variation	8.91%	5.41%			

GROWTH SURVEY — BODY

Litter Ref. CL/128 and CL/79 — Age: 6 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	12.76	7.36	3.164	0.2356	0.0320
	Range	10.5 - 17.7	7.0 - 8.0			
Male	S.E. of Mean	1.2663	0.1691			
	Coeff. of Variation	22.19%	5.14%			
	Mean	16.14	7.80	3.096	0.2653	0.0340
	Range	12.1 - 17.8	7.2 - 8.0			
Female	S.E. of Mean	0.9368	0.1217			
	Coeff. of Variation	15.36%	4.12%			
	Mean	14.73	7.62	3.124	0.2537	0.0333
	Range	10.5 - 17.8	7.0 - 8.0			
Combination	S.E. of Mean	0.8804	0.1153			
	Coeff. of Variation	20.70%	5.24%			

GROWTH SURVEY — BODY

Litter Ref. CL/81 and CL/121 — Age: 7 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	19.43	8.23	3.062	0.2869	0.0349
	Range	18.7 - 20.2	8.0 - 8.4			
	S.E. of Mean	0.4334	0.1204			
	Coeff. of Variation	3.86%	2.53%			
Female	Mean	19.03	8.10	3.034	0.2900	0.0358
	Range	18.1 - 20.4	7.9 - 8.3			
	S.E. of Mean	0.2494	0.0469			
	Coeff. of Variation	3.93%	1.75%			
Combination	Mean	19.13	8.13	3.041	0.2894	0.0356
	Range	18.1 - 20.4	7.9 - 8.4			
	S.E. of Mean	0.2126	0.0469			
	Coeff. of Variation	3.85%	1.99%			

GROWTH SURVEY — BODY

Litter Ref. CL/82 and CL/122 — Age: 8 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	17.00	7.79	3.031	0.2801	0.0360
	Range	15.5 - 19.3	7.6 - 8.0			
	S.E. of Mean	0.4005	0.0436			
	Coeff. of Variation	6.66%	1.60%			
Female	Mean	16.10	7.68	3.039	0.2730	0.0355
	Range	15.6 - 17.0	7.6 - 7.8			
	S.E. of Mean	0.3189	0.0480			
	Coeff. of Variation	3.96%	1.25%			
Combination	Mean	16.70	7.75	3.034	0.2780	0.0359
	Range	15.5 - 19.3	7.6 - 8.0			
	S.E. of Mean	0.3059	0.0361			
	Coeff. of Variation	6.35%	1.61%			

GROWTH SURVEY — BODY

Litter Ref. CL/84 and CL/85 — Age: 9 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	20.50	8.73	3.195	0.2690	0.0308
	Range	16.4 - 22.7	8.1 - 9.1			
	S.E. of Mean	0.9658	0.1382			
	Coeff. of Variation	11.54%	3.88%			
Female	Mean	19.28	8.37	3.127	0.2752	0.0329
	Range	15.3 - 21.9	8.1 - 8.6			
	S.E. of Mean	0.9734	0.0806			
	Coeff. of Variation	12.37%	2.35%			
Combination	Mean	19.89	8.55	3.161	0.2721	0.0318
	Range	15.3 - 22.7	8.1 - 9.1			
	S.E. of Mean	0.6790	0.0943			
	Coeff. of Variation	11.83%	3.82%			

GROWTH SURVEY — BODY

Litter Ref. CL/86 and CL/87 — Age: 10 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	23.39	8.70	3.044	0.3090	0.0355
	Range	21.1 - 26.3	8.4 - 9.2			
	S.E. of Mean	0.7278	0.1114			
	Coeff. of Variation	8.23%	3.38%			
Female	Mean	23.12	8.68	3.048	0.3069	0.0354
	Range	20.9 - 26.1	8.1 - 9.2			
	S.E. of Mean	0.9578	0.2083			
	Coeff. of Variation	9.26%	5.37%			
Combination	Mean	23.28	8.69	3.045	0.3083	0.0355
	Range	20.9 - 26.3	8.1 - 9.2			
	S.E. of Mean	0.5560	0.1025			
	Coeff. of Variation	8.27%	4.09%			

GROWTH SURVEY — BODY

Litter Ref. CL/88 and CL/89 — Age: 11 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	25.43	9.07	3.084	0.3091	0.0341
	Range	24.5 - 26.4	8.8 - 9.3			
	S.E. of Mean	0.2789	0.0922			
	Coeff. of Variation	2.69%	2.48%			
Female	Mean	25.38	9.03	3.075	0.3113	0.0345
	Range	23.4 - 26.6	5.7 - 9.3			
	S.E. of Mean	0.4792	0.0954			
	Coeff. of Variation	4.62%	2.59%			
Combination	Mean	25.41	9.05	3.079	0.3102	0.0343
	Range	23.4 - 26.6	8.7 - 9.3			
	S.E. of Mean	0.2644	0.0632			
	Coeff. of Variation	3.61%	2.43%			

GROWTH SURVEY — BODY

Litter Ref. CL/94 and CL/95 — Age: 12 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	27.98	9.56	3.153	0.3061	0.0320
	Range	26.0 - 29.3	9.3 - 9.9			
	S.E. of Mean	0.4704	0.0755			
	Coeff. of Variation	4.76%	2.23%			
Female	Mean	27.80	9.58	3.162	0.3029	0.0316
	Range	27.1 - 29.4	9.5 - 9.7			
	S.E. of Mean	0.5401	0.0480			
	Coeff. of Variation	3.89%	1.00%			
Combination	Mean	27.92	9.57	3.155	0.3049	0.0319
	Range	26.0 - 29.4	9.3 - 9.9			
	S.E. of Mean	0.3479	0.0510			
	Coeff. of Variation	4.32%	1.85%			

GROWTH SURVEY — BODY

Litter Ref. CL/96 and CL/124 — Age: 13 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	32.33	9.95	3.123	0.3266	0.0328
	Range	30.4 - 34.1	9.5 - 10.8			
	S.E. of Mean	0.4766	0.1453			
	Coeff. of Variation	4.17%	4.13%			
Female	Mean	32.50	9.85	3.088	0.3350	0.0340
	Range	31.0 - 34.3	9.7 - 10.0			
	S.E. of Mean	0.6868	0.0866			
	Coeff. of Variation	4.23%	1.76%			
Combination	Mean	32.38	9.92	3.111	0.3290	0.0332
	Range	30.4 - 34.3	9.5 - 10.8			
	S.E. of Mean	0.3739	0.0990			
	Coeff. of Variation	4.00%	3.46%			

GROWTH SURVEY — BODY

Litter Ref. CL/98 and CL/99 — Age: 14 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	36.39	10.21	3.084	0.3491	0.0342
	Range	31.7 - 39.5	9.5 - 10.7			
	S.E. of Mean	0.9711	0.1792			
	Coeff. of Variation	7.06%	4.64%			
Female	Mean	32.10	9.74	3.065	0.3384	0.0347
	Range	30.7 - 36.4	9.3 - 10.0			
	S.E. of Mean	1.1041	0.1208			
	Coeff. of Variation	7.69%	2.77%			
Combination	Mean	34.60	10.02	3.076	0.3446	0.0344
	Range	30.7 - 39.5	9.3 - 10.7			
	S.E. of Mean	0.9437	0.1319			
	Coeff. of Variation	9.45%	4.56%			

GROWTH SURVEY — BODY

Litter Ref. CL/100 and CL/101 — Age: 15 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	41.94	10.66	3.067	0.3691	0.0346
	Range	38.4 - 44.0	10.3 - 11.1			
	S.E. of Mean	0.9569	0.1288			
	Coeff. of Variation	5.10%	2.70%			
Female	Mean	38.49	10.69	3.165	0.3368	0.0315
	Range	34.3 - 43.1	10.2 - 11.4			
	S.E. of Mean	1.0754	0.1640			
	Coeff. of Variation	7.39%	4.06%			
Combination	Mean	39.93	10.68	3.124	0.3501	0.0328
	Range	34.3 - 44.0	10.2 - 11.4			
	S.E. of Mean	0.8777	0.1054			
	Coeff. of Variation	7.61%	3.41%			

Litter Ref. CL/102 and CL/103 Age: 16 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	40.43	10.93	3.186	0.3384	0.0310
	Range	36.3 - 43.0	10.4 - 11.4			
Male	S.E. of Mean	2.0867	0.2907			
	Coeff. of Variation	8.94%	4.61%			
	Mean	38.11	10.42	3.099	0.3510	0.0377
	Range	32.3 - 41.4	9.9 - 10.9			
Female	S.E. of Mean	1.1458	0.1114			
	Coeff. of Variation	9.02%	3.21%			
	Mean	38.69	10.55	3.121	0.3476	0.0329
	Range	32.2 - 43.0	9.9 - 11.4			
Combination	S.E. of Mean	1.0030	0.1229			
	Coeff. of Variation	8.98%	4.03%			

GROWTH SURVEY — BODY

Litter Ref. CL/104 and CL/125 — Age: 17 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	47.67	11.61	3.205	0.3537	0.0305
	Range	46.2 - 49.8	11.3 - 11.9			
	S.E. of Mean	0.4902	0.0735			
	Coeff. of Variation	2.72%	1.68%			
Female	Mean	45.10	11.40	3.207	0.3470	0.0304
	Range	39.3 - 49.0	11.0 - 12.0			
	S.E. of Mean	1.7073	0.1817			
	Coeff. of Variation	8.46%	3.56%			
Combination	Mean	46.40	11.53	3.206	0.3505	0.0304
	Range	39.3 - 49.8	11.0 - 12.0			
	S.E. of Mean	0.8150	0.0883			
	Coeff. of Variation	6.06%	2.65%			

GROWTH SURVEY

Litter Ref. CL/106 and CL/107 — Age: 18 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	43.37	11.47	3.273	0.3297	0.0287
	Range	38.5 - 50.5	11.2 - 11.8			
Male	S.E. of Mean	3.6448	0.1764			
	Coeff. of Variation	14.56%	2.66%			
	Mean	43.92	11.42	3.243	0.3368	0.0295
	Range	36.8 - 48.2	10.9 - 11.9			
Female	S.E. of Mean	1.4071	0.1114			
	Coeff. of Variation	9.61%	2.93%			
	Mean	43.78	11.43	3.250	0.3351	0.0293
	Range	36.8 - 50.5	10.9 - 11.9			
Combination	S.E. of Mean	1.2997	0.0906			
	Coeff. of Variation	10.28%	2.75%			

GROWTH SURVEY BODY

Litter Ref. CL/108 and CL/109 Age: 19 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	43.88	11.58	3.342	0.3272	0.0283
	Range	39.0 - 49.0	11.4 - 12.4			
Male	S.E. of Mean	2.1266	0.3119			
	Coeff. of Variation	9.69%	5.39%			
	Mean	44.48	11.44	3.234	0.3399	0.0297
	Range	36.3 - 50.5	11.0 - 11.9			
Female	S.E. of Mean	1.6996	0.1100			
	Coeff. of Variation	10.81%	2.72%			
	Mean	44.28	11.48	3.270	0.3360	0.0293
	Range	36.3 - 50.5	11.0 - 12.4			
Combination	S.E. of Mean	1.2822	0.1200			
	Coeff. of Variation	10.03%	3.62%			

GROWTH SURVEY — BODY

Litter Ref. CL/110 and CL/111 — Age: 20 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	54.20	12.36	3.272	0.3548	0.0297
	Range	47.6 - 59.0	11.8 - 12.7			
Male	S.E. of Mean	2.3839	0.1568			
	Coeff. of Variation	9.84%	2.84%			
	Mean	50.39	11.94	3.239	0.3535	0.0296
	Range	44.7 - 56.5	11.2 - 12.4			
Female	S.E. of Mean	2.0420	0.1811			
	Coeff. of Variation	10.72%	4.01%			
	Mean	51.98	12.12	3.253	0.3539	0.0292
	Range	44.7 - 59.0	11.2 - 12.7			
Combination	S.E. of Mean	1.5841	0.1342			
	Coeff. of Variation	10.56%	3.84%			

GROWTH SURVEY — BODY

Litter Ref. CL/112 and CL/113 — Age: 21 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	55.42	12.76	3.349	0.3404	0.0267
	Range	53.2 - 59.5	12.4 - 13.4			
	S.E. of Mean	1.1088	0.1913			
	Coeff. of Variation	4.47%	3.35%			
Female	Mean	53.14	12.60	3.355	0.3347	0.0266
	Range	48.3 - 58.7	12.2 - 13.2			
	S.E. of Mean	1.3443	0.1292			
	Coeff. of Variation	6.69%	2.71%			
Combination	Mean	54.09	12.67	3.353	0.3369	0.0266
	Range	48.3 - 59.5	12.2 - 13.4			
	S.E. of Mean	0.9358	0.1068			
	Coeff. of Variation	5.99%	2.92%			

GROWTH SURVEY

BODY

Litter Ref. CL/114

Age: 22 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	39.67	11.03	3.235	0.3261	0.0196
	Range	38.0 - 42.5	10.8 - 11.4			
	S.E. of Mean	1.4240	0.1857			
	Coeff. of Variation	6.22%	2.92%			
Female	Mean	38.20	10.63	3.158	0.3381	0.0318
	Range	37.4 - 39.0	10.6 - 10.7			
	S.E. of Mean	0.4618	0.0332			
	Coeff. of Variation	2.09%	0.55%			

GROWTH SURVEY — BODY

Litter Ref. CL/148 — Age: 23 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	53.55	11.20	2.972	0.4269	0.0381
	Range	50.0 - 57.1	10.9 - 11.5			
	S.E. of Mean	3.5500	0.3000			
	Coeff. of Variation	9.38%	3.79%			
Female	Mean	53.88	11.13	2.945	0.4349	0.0391
	Range	51.3 - 56.2	11.0 - 11.5			
	S.E. of Mean	1.1750	0.1249			
	Coeff. of Variation	4.36%	2.25%			

GROWTH SURVEY — BODY

Litter Ref. CL/150 — Age: 24 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	58.28	12.54	3.236	0.3706	0.0296
	Range	52.5 - 63.1	12.1 - 13.2			
	S.E. of Mean	2.0908	0.1833			
	Coeff. of Variation	8.02%	3.27%			
Female	Mean	55.2	11.9	3.130	0.3898	0.0328
	Range	-	-			
	S.E. of Mean	-	-			
	Coeff. of Variation	-	-			

GROWTH SURVEY — BODY

Litter Ref. CL/127 — Age: 25 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	61.50	12.45	3.154	0.3968	0.0319
	Range	55.0 - 65.3	11.9 - 13.0			
	S.E. of Mean	2.2446	0.2328			
	Coeff. of Variation	7.30%	3.74%			
Female	Mean	60.80	12.15	3.092	0.4119	0.0339
	Range	59.5 - 62.1	12.0 - 12.3			
	S.E. of Mean	1.3000	0.1500			
	Coeff. of Variation	3.02%	1.75%			

GROWTH SURVEY — BODY

Litter Ref. CL/136 — Age: 26 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	84.63	14.20	3.237	0.4197	0.0296
	Range	79.0 - 87.7	14.0 - 14.3			
	S.E. of Mean	2.8203	0.1000			
	Coeff. of Variation	5.77%	1.21%			
Female	Mean	80.37	13.83	3.204	0.4202	0.0304
	Range	78.3 - 82.0	13.5 - 14.0			
	S.E. of Mean	1.0898	0.1664			
	Coeff. of Variation	2.34%	2.08%			

GROWTH SURVEY

BODY

Litter Ref. CL/141

Age: 27 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	74.90	13.4	3.176	0.4171	0.0311
	Range	74.90	13.4			
	S.E. of Mean	-	-			
	Coeff. of Variation	-	-			
Female	Mean	69.36	13.00	3.168	0.4104	0.0316
	Range	58.5 - 76.2	12.0 - 13.5			
	S.E. of Mean	3.0889	0.2738			
	Coeff. of Variation	9.95%	4.71%			

GROWTH SURVEY — BODY

Litter Ref. CL/134 — Age: 28 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	73.0	13.67	3.266	0.3906	0.0286
	Range	70.3 - 75.7	13.6 - 13.8			
	S.E. of Mean	1.5588	0.0671			
	Coeff. of Variation	3.70%	0.85%			
Female	Mean	75.77	13.70	3.238	0.4037	0.0295
	Range	74.7 - 76.4	13.6 - 13.9			
	S.E. of Mean	0.5365	0.1000			
	Coeff. of Variation	1.23%	1.26%			

Litter Ref. CL/145 Age: 29 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	79.33	14.18	3.301	0.3945	0.0278
	Range	73.6 - 84.8	13.7 - 14.6			
	S.E. of Mean	2.6650	0.2214			
	Coeff. of Variation	6.72%	3.12%			
Female	Mean	72.90	13.70	3.285	0.3884	0.0284
	Range	66.7 - 79.1	13.3 - 14.1			
	S.E. of Mean	6.2000	0.4000			
	Coeff. of Variation	12.03%	4.13%			

GROWTH SURVEY — BODY

Litter Ref. CL/135 — Age: 30 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	97.15	15.00	3.263	0.4318	0.0288
	Range	96.1 - 98.2	15.0			
	S.E. of Mean	1.0500	0.0000			
	Coeff. of Variation	1.53%	0.00%			
Female	Mean	92.4	14.45	3.197	0.4425	0.0306
	Range	87.3 - 96.3	14.1 - 14.7			
	S.E. of Mean	2.0921	0.1257			
	Coeff. of Variation	4.53%	1.74%			

GROWTH SURVEY

BODY

Litter Ref. CL/143

Age: 31 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	86.93	13.85	3.139	0.4532	0.0327
	Range	76.1 - 97.1	13.3 - 14.4			
	S.E. of Mean	4.5837	0.2328			
	Coeff. of Variation	10.55%	3.36%			
Female	Mean	85.30	14.05	3.189	0.4321	0.0308
	Range	84.3 - 86.3	13.9 - 14.2			
	S.E. of Mean	1.0000	0.1500			
	Coeff. of Variation	1.66%	1.51%			

GROWTH SURVEY

BODY

Litter Ref. CL/147

Age: 32 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	75.93	13.37	3.154	0.4248	0.0318
	Range	74.0 - 77.8	13.1 - 13.8			
	S.E. of Mean	1.0975	0.2186			
	Coeff. of Variation	2.50%	2.83%			
Female	Mean	72.90	13.47	3.228	0.4018	0.0298
	Range	70.1 - 77.1	13.1 - 14.0			
	S.E. of Mean	2.1385	0.2729			
	Coeff. of Variation	5.08%	3.51%			

GROWTH SURVEY — BODY

Litter Ref. CL/137 — Age: 33 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	115.7	16.10	3.304	0.4464	0.0277
	Range	113.5 - 117.9	16.0 - 16.2			
	S.E. of Mean	2.2000	0.1000			
	Coeff. of Variation	2.69%	0.88%			
Female	Mean	104.43	15.47	3.285	0.4364	0.0282
	Range	100.1 - 111.1	15.3 - 15.8			
	S.E. of Mean	3.3830	0.1667			
	Coeff. of Variation	5.61%	1.87%			

GROWTH SURVEY — BODY

Litter Ref. CL/129 — Age: 34 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	100.35	14.70	3.165	0.4644	0.0316
	Range	92.5 - 108.2	14.3 - 15.1			
	S.E. of Mean	7.8500	0.4000			
	Coeff. of Variation	11.06%	3.85%			
Female	Mean	85.48	13.63	3.100	0.4601	0.0338
	Range	62.2 - 98.6	12.5 - 14.6			
	S.E. of Mean	7.9891	0.4956			
	Coeff. of Variation	18.69%	7.27%			

GROWTH SURVEY — BODY

Litter Ref. CL/146 — Age: 35 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	101.27	14.03	3.008	0.5145	0.0367
	Range	97.5 - 107.5	13.6 - 14.5			
	S.E. of Mean	3.1392	0.2604			
	Coeff. of Variation	5.37%	3.21%			
Female	Mean	93.70	13.77	3.032	0.4942	0.0359
	Range	89.2 - 101.8	13.4 - 14.4			
	S.E. of Mean	4.6583	0.3180			
	Coeff. of Variation	7.50%	4.00%			

GROWTH SURVEY — BODY

Litter Ref. CL/132 — Age: 36 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	139.20	16.33	3.152	0.5220	0.0320
	Range	133.0 - 142.3	16.1 - 16.5			
	S.E. of Mean	3.1000	0.1204			
	Coeff. of Variation	3.86%	1.28%			
Female	Mean	117.17	15.30	3.125	0.5010	0.0327
	Range	115.1 - 119.6	15.2 - 15.5			
	S.E. of Mean	1.3017	0.1000			
	Coeff. of Variation	1.92%	1.13%			

GROWTH SURVEY — BODY

Litter Ref. CL/138 — Age: 37 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	117.13	15.80	3.232	0.4692	0.0297
	Range	109.3 - 132.7	15.4 - 16.5			
	S.E. of Mean	7.7834	0.3511			
	Coeff. of Variation	11.51%	3.85%			
Female	Mean	105.13	14.97	3.174	0.4691	0.0313
	Range	99.8 - 108.3	14.6 - 15.5			
	S.E. of Mean	2.6823	0.2729			
	Coeff. of Variation	4.42%	3.16%			

GROWTH SURVEY — BODY

Litter Ref. CL/131 — Age: 38 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	138.47	16.27	3.144	0.5231	0.0322
	Range	134.5 - 141.5	16.1 - 16.4			
	S.E. of Mean	2.0739	0.0883			
	Coeff. of Variation	2.59%	0.94%			
Female	Mean	109.33	15.43	3.228	0.4592	0.0298
	Range	105.4 - 112.0	15.4 - 15.5			
	S.E. of Mean	2.0078	0.0332			
	Coeff. of Variation	3.18%	0.38%			

GROWTH SURVEY

BODY

Litter Ref. CL/130

Age: 39 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	157.40	17.50	3.241	0.5140	0.0294
	Range	155.7 - 158.7	17.2 - 17.7			
	S.E. of Mean	0.8888	0.1526			
	Coeff. of Variation	0.98%	1.51%			
Female	Mean	133.33	16.60	3.249	0.4839	0.0291
	Range	131.0 - 137.4	16.4 - 16.8			
	S.E. of Mean	2.0407	0.1153			
	Coeff. of Variation	2.65%	1.20%			

GROWTH SURVEY — BODY

Litter Ref. CL/119 — Age: 40 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	115.58	16.98	3.485	0.4009	0.0236
	Range	113.7 - 117.4	16.8 - 17.1			
	S.E. of Mean	0.7941	0.0632			
	Coeff. of Variation	1.37%	0.74%			
Female	Mean	104.30	15.85	3.367	0.4152	0.0262
	Range	102.0 - 106.6	15.3 - 16.4			
	S.E. of Mean	2.3000	0.5500			
	Coeff. of Variation	3.12%	4.91%			

GROWTH SURVEY

EXPERIMENTAL

FIRST SAMPLE

BIRTH - 20 DAYS

Litter Ref. A/EL 46 Age: BIRTH

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	5.73	4.86	2.716	0.2426	0.0499
	Range	5.2 - 6.2	4.7 - 5.0			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	5.88	4.86	2.797	0.2489	0.0512
	Range	5.4 - 6.4	4.7 - 5.0			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	5.81	4.8	2.756	0.2470	0.0525
	Range	5.2 - 6.4	4.7 - 5.0			
	S.E. of Mean	0.0241	0.0244			
	Coeff. of Variation	5.2%	2.4%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 30 — Age: 1 DAY

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	6.1	5.4	2.974	0.2092	0.0387
	Range	5.1 - 7.0	4.4 - 5.8			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	5.7	5.3	2.997	0.2029	0.0383
	Range	3.9 - 6.4	5.0 - 5.6			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	5.9	5.3	2.986	0.2387	0.0396
	Range	3.9 - 7.0	4.4 - 5.8			
	S.E. of Mean	0.1671	0.0261			
	Coeff. of Variation	11.3%	6.2%			

Litter Ref. A/EL 51 Age: 2 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	6.2	5.7	3.117	0.1908	0.0335
	Range	5.3 - 8.2	5.0 - 6.3			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	5.9	5.7	3.162	0.1816	0.0319
	Range	5.1 - 7.2	5.0 - 6.6			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	6.09	5.7	3.138	0.1855	0.0329
	Range	5.1 - 8.2	5.0 - 6.6			
	S.E. of Mean	0.2177	0.1200			
	Coeff. of Variation	13.8%	8.1%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 49 — Age: 3 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	7.8	6.3	3.181	0.1965	0.0312
	Range	5.9 - 8.8	5.4 - 8.1			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	7.8	6.1	3.105	0.2096	0.0344
	Range	5.9 - 8.7	5.7 - 6.5			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	7.8	6.2	3.141	0.2008	0.0327
	Range	5.9 - 8.8	5.4 - 8.1			
	S.E. of Mean	0.2297	0.4395			
	Coeff. of Variation	12.8%	9.2%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 42 — Age: 4 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	8.0	5.9	2.966	0.2298	0.0390
	Range	7.0 - 9.5	5.7 - 6.3			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	7.9	5.9	3.004	0.2269	0.0385
	Range	6.2 - 9.1	5.5 - 6.3			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	7.95	5.9	2.985	0.2253	0.0387
	Range	6.2 - 9.5	5.5 - 6.3			
	S.E. of Mean	0.2431	0.0228			
	Coeff. of Variation	12.5%	5.1%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 41 — Age: 5 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	8.4	6.5	3.223	0.1988	0.0306
	Range	7.7 - 10.1	6.1 - 6.8			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	8.4	6.6	3.280	0.1928	0.0292
	Range	6.4 - 10.2	5.9 - 7.3			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	8.4	6.6	3.253	0.1928	0.0292
	Range	6.4 - 10.2	5.9 - 7.3			
	S.E. of Mean	0.2402	0.0753			
	Coeff. of Variation	3.7%	4.7%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 8 — Age: 6 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	10.95	6.3	2.845	0.2759	0.0438
	Range	9.9 - 12.5	6.1 - 6.5			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	10.4	6.2	2.840	0.2706	0.0436
	Range	9.9 - 12.1	6.1 - 6.4			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	10.7	6.2	2.842	0.2722	0.0499
	Range	9.9 - 12.5	6.1 - 6.5			
	S.E. of Mean	0.2084	0.0114			
	Coeff. of Variation	7.5%	2.2%			

GROWTH SURVEY BODY

Litter Ref. A/EL 9 Age: 7 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	10.28	6.37	2.922	0.2533	0.0398
	Range	9.2 - 12.2	6.0 - 6.8			
	S,E. of Mean					
	Coeff. of Variation					
Female	Mean	9.86	6.26	2.924	0.2516	0.0402
	Range	8.9 - 11.4	6.0 - 6.5			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	10.05	6.3	2.926	0.2524	0.0403
	Range	8.9 - 12.2	6.0 - 6.8			
	S.E. of Mean	0.2232	0.0538			
	Coeff. of Variation	9.4%	3.6%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 35 — Age: 8 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	11.8	6.9	3.044	0.2478	0.0359
	Range	10.3 - 13.2	6.6 - 7.2			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	10.9	6.8	3.091	0.2356	0.0347
	Range	9.5 - 12.1	6.5 - 7.2			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	11.3	6.8	3.067	0.2395	0.0359
	Range	9.5 - 13.2	6.5 - 7.2			
	S,E. of Mean	0.2665	0.0575			
	Coeff. of Variation	9.1%	10.2%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 36 — Age: 9 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	10.9	7.1	3.201	0.2162	0.0305
	Range	9.3 - 13.6	6.8 - 7.5			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	10.4	7.0	3.201	0.2122	0.0303
	Range	9.0 - 12.2	6.7 - 7.4			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	10.7	7.0	3.217	0.2139	0.0312
	Range	9.0 - 13.6	6.7 - 7.5			
	S.E. of Mean	0.3435	0.0583			
	Coeff. of Variation	12.4%	3.2%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 1 — Age: 10 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	15.95	7.42	3.004	0.2897	0.0390
	Range	14.6 - 18.0	7.34 - 8.05			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	13.88	7.40	3.058	0.2535	0.0343
	Range	9.2 - 18.2	6.5 - 7.9			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	14.9	7.4	3.038	0.2668	0.0368
	Range	9.2 - 18.2	6.5 - 8.05			
	S.E. of Mean	0.6088	0.0938			
	Coeff. of Variation	18.1%	5.5%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 37 — Age: 11 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	15.1	7.6	3.102	0.2614	0.0344
	Range	13.0 - 16.3	7.3 - 7.9			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	15.2	7.7	3.130	0.2564	0.0333
	Range	13.1 - 16.4	7.5 - 8.0			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	15.1	7.7	3.118	0.2549	0.0331
	Range	13.0 - 16.4	7.3 - 8.0			
	S.E. of Mean	0.2642	0.0134			
	Coeff. of Variation	7.0%	2.7%			

Litter ref. A/EL 38 Age: 12 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	17.0	8.1	3.141	0.2591	0.0320
	Range	15.8 - 18.2	7.8 - 8.4			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	16.7	8.1	3.141	0.2545	0.0314
	Range	15.9 - 19.1	7.2 - 8.4			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	16.9	8.1	3.141	0.2608	0.0318
	Range	15.8 - 19.1	7.2 - 8.4			
	S.E. of Mean	0.4431	0.0253			
	Coeff. of Variation	10.1%	3.8%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 10 — Age: 13 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	21.65	8.42	3.025	0.3189	0.0363
	Range	18.3 - 27.2	8.1 - 8.9			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	19.61	8.15	3.470	0.2952	0.0400
	Range	17.4 - 22.0	7.6 - 8.5			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	20.6	8.3	3.208	0.2990	0.0360
	Range	17.4 - 27.2	7.6 - 8.9			
	S.E. of Mean	0.5724	0.0241			
	Coeff. of Variation	11.3%	3.8%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 40 — Age: 14 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	14.1	7.6	3.158	0.2441	0.0321
	Range	12.5 - 15.9	7.4 - 7.9			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	13.4	7.5	3.164	0.2382	0.0318
	Range	10.9 - 15.5	7.1 - 8.1			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	13.7	7.5	3.161	0.2399	0.0325
	Range	10.9 - 15.9	7.1 - 8.1			
	S.E. of Mean	0.3402	0.0225			
	Coeff. of Variation	9.6%	3.7%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 2 — Age: 15 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	17.49	8.24	3.095	0.2576	0.0313
	Range	15.9 - 19.2	7.6 - 8.5			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	15.28	7.52	3.114	0.2702	0.0359
	Range	13.35 - 16.4	7.4 - 8.5			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	16.3	7.7	3.104	0.2639	0.0357
	Range	13.35 - 19.2	7.4 - 8.5			
	S.E. of Mean	0.3975	0.0275			
	Coeff. of Variation	10.2%	4.7%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 20 — Age: 16 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	23.1	9.02	3.172	0.2839	0.0315
	Range	19.8 - 26.2	8.7 - 9.6			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	22.58	8.95	3.167	0.2819	0.0315
	Range	20.1 - 25.0	8.6 - 9.3			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	22.84	8.9	3.170	0.2829	0.0324
	Range	19.8 - 26.2	8.6 - 9.6			
	S.E. of Mean	0.6243	0.1081			
	Coeff. of Variation	9.9%	4.3%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 17 — Age: 17 DAYS

	Analysis	Weight (Cms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	13.11	8.92	3.134	0.1648	0.0185
	Range	20.4 - 26.2	8.3 - 9.5			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	21.1	8.68	3.140	0.2801	0.0323
	Range	19.4 - 23.3	8.3 - 9.2			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	22.06	8.8	3.137	0.2861	0.0324
	Range	19.7 - 26.2	8.3 - 9.5			
	S.E. of Mean	0.4954	0.0889			
	Coeff. of Variation	8.6%	3.9%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 19 — Age: 18 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	24.23	9.35	3.233	0.2772	0.0296
	Range	22.4 - 25.8	9.0 - 9.9			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	23.23	9.03	3.199	0.2849	0.0315
	Range	18.5 - 26.8	8.1 - 9.6			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	23.73	9.2	3.204	0.2806	0.0305
	Range	18.5 - 26.8	8.1 - 9.9			
	S.E. of Mean	0.5489	0.3225			
	Coeff. of Variation	9.2%	4.3%			

Litter Ref. A/EL 15 Age: 19 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	23.22	9.13	3.214	0.2786	0.0305
	Range	21.6 - 24.9	8.9 - 9.5			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	23.78	9.33	3.245	0.2732	0.0293
	Range	22.7 - 25.0	9.2 - 9.6			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	23.5	9.2	3.228	0.2745	0.0302
	Range	21.6 - 25.0	8.9 - 9.6			
	S.E. of Mean	0.2757	0.0182			
	Coeff. of Variation	4.2%	2.2%			

GROWTH SURVEY — BODY

Litter Ref. A/EL 55 — Age: 20 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	25.2	9.5	3.266	0.2892	0.0294
	Range	20.9 - 28.7	9.1 - 10.4			
	S.E. of Mean					
	Coeff. of Variation					
Female	Mean	24.0	9.3			
	Range	21.8 - 26.1	9.1 - 9.6			
	S.E. of Mean					
	Coeff. of Variation					
Combination	Mean	24.7	9.4	3.258	0.2745	0.0297
	Range	20.9 - 28.7	9.1 - 10.4			
	S.E. of Mean	0.5656	0.0310			
	Coeff. of Variation	8.6%	3.9%			

GROWTH SURVEY

EXPERIMENTAL

SECOND SAMPLE

1 - 40 DAYS

GROWTH SURVEY — BODY

Litter Ref. EL/138 — Age: 1 DAY

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	5.16	5.24	3.035	0.1879	0.0359
	Range	4.6 - 5.6	5.0 - 5.6			
Male	S.E. of Mean	0.1025	0.0656			
	Coeff. of Variation	6.28%	3.94%			
	Mean	5.28	5.26	3.019	0.1908	0.0363
	Range	5.1 - 5.5	5.0 - 5.5			
Female	S.E. of Mean	0.0800	0.0648			
	Coeff. of Variation	4.01%	3.27%			
	Mean	5.21	5.25	3.029	0.1890	0.0360
	Range	4.6 - 5.6	5.0 - 5.6			
Combination	S.E. of Mean	0.0686	0.0458			
	Coeff. of Variation	5.43%	3.57%			

GROWTH SURVEY — BODY

Litter Ref. EL/148 — Age: 2 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	6.39	5.70	3.072	0.1967	0.0345
	Range	6.1 - 6.6	6.1 - 6.6			
	S.E. of Mean	0.0707	0.0436			
	Coeff. of Variation	2.92%	2.02%			
Female	Mean	5.67	5.44	3.056	0.1969	0.0352
	Range	5.2 - 6.2	5.3 - 5.7			
	S.E. of Mean	0.1054	0.0480			
	Coeff. of Variation	5.58%	2.62%			
Combination	Mean	5.98	5.56	3.063	0.1934	0.0348
	Range	5.2 - 6.6	5.3 - 5.8			
	S.E. of Mean	0.1127	0.0458			
	Coeff. of Variation	7.54%	3.28%			

GROWTH SURVEY — BODY

Litter Ref. EL/125 — Age: 3 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	6.85	5.80	3.057	0.2036	0.0351
	Range	6.5 - 7.6	5.6 - 6.2			
	S.E. of Mean	0.1131	0.0500			
	Coeff. of Variation	5.48%	2.88%			
Female	Mean	6.72	6.05	3.208	0.1836	0.0303
	Range	6.4 - 7.3	5.9 - 6.3			
	S.E. of Mean	0.1493	0.0721			
	Coeff. of Variation	5.44%	2.91%			
Combination	Mean	6.80	5.89	3.110	0.1960	0.0333
	Range	6.4 - 7.6	5.6 - 6.3			
	S.E. of Mean	0.0889	0.0500			
	Coeff. of Variation	5.38%	3.50%			

GROWTH SURVEY — BODY

Litter Ref. EL/100 — Age: 4 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	8.88	6.94	3.365	0.1844	0.0266
	Range	6.8 - 9.6	6.5 - 7.4			
	S.E. of Mean	0.3055	0.0900			
	Coeff. of Variation	10.32%	3.89%			
Female	Mean	8.07	6.62	3.316	0.1841	0.0278
	Range	5.7 - 9.5	6.1 - 6.8			
	S.E. of Mean	0.5959	0.1077			
	Coeff. of Variation	18.09%	3.99%			
Combination	Mean	8.55	6.81	3.346	0.1844	0.0271
	Range	5.7 - 9.6	6.1 - 7.4			
	S.E. of Mean	0.3066	0.0794			
	Coeff. of Variation	13.89%	4.51%			

GROWTH SURVEY — BODY

Litter Ref. EL/115 — Age: 5 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	11.25	7.09	3.180	0.2238	0.0316
	Range	6.7 - 13.1	6.3 - 7.5			
	S.E. of Mean	0.6136	0.1253			
	Coeff. of Variation	17.25%	5.58%			
Female	Mean	11.28	6.96	3.106	0.2329	0.0335
	Range	10.3 - 13.0	6.6 - 7.2			
	S.E. of Mean	0.4543	0.1030			
	Coeff. of Variation	9.01%	3.31%			
Combination	Mean	11.26	7.05	3.156	0.2265	0.0321
	Range	6.7 - 13.1	6.3 - 7.5			
	S.E. of Mean	0.4254	0.0894			
	Coeff. of Variation	14.63%	4.91%			

GROWTH SURVEY

BODY

Litter Ref. EL/101

Age: 6 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	11.92	7.52	3.295	0.2108	0.0280
	Range	10.4 - 13.8	6.9 - 8.0			
	S.E. of Mean	0.2909	0.1114			
	Coeff. of Variation	7.72%	4.68%			
Female	Mean	11.88	7.34	3.222	0.2205	0.0300
	Range	9.6 - 13.0	7.1 - 7.6			
	S.E. of Mean	0.6184	0.1077			
	Coeff. of Variation	11.64%	3.28%			
Combination	Mean	11.91	7.46	3.270	0.2140	0.0287
	Range	9.6 - 13.8	6.9 - 8.0			
	S.E. of Mean	0.2696	0.0831			
	Coeff. of Variation	8.77%	4.32%			

GROWTH SURVEY — BODY

Litter Ref. EL/119 — Age: 7 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	13.72	7.52	3.516	0.1242	0.0323
	Range	10.4 - 16.0	7.0 - 8.0			
	S.E. of Mean	0.6227	0.0985			
	Coeff. of Variation	13.62%	3.92%			
Female	Mean	14.02	7.60	3.158	0.2427	0.0319
	Range	11.7 - 16.4	7.2 - 7.9			
	S.E. of Mean	0.6785	0.1000			
	Coeff. of Variation	11.85%	3.22%			
Combination	Mean	13.84	7.55	3.153	0.2428	0.0322
	Range	10.4 - 16.4	7.0 - 8.0			
	S.E. of Mean	0.4474	0.0693			
	Coeff. of Variation	12.52%	3.57%			

GROWTH SURVEY BODY

Litter Ref. EL/114 Age: 8 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	14.57	7.71	3.520	0.2481	0.0318
	Range	9.6 - 17.7	7.1 - 8.5			
Male	S.E. of Mean	0.7656	0.1439			
	Coeff. of Variation	15.76%	5.60%			
	Mean	13.87	7.47	3.127	0.2486	0.0333
	Range	7.1 - 16.1	5.9 - 8.0			
Female	S.E. of Mean	1.3837	0.3294			
	Coeff. of Variation	24.44%	10.80%			
	Mean	14.29	7.61	3.152	0.2468	0.0324
	Range	7.1 - 17.7	5.9 - 8.5			
Combination	S.E. of Mean	0.6948	0.1536			
	Coeff. of Variation	18.83%	7.82%			

GROWTH SURVEY — BODY

Litter Ref. EL/116 — Age: 9 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	14.75	7.85	3.202	0.2394	0.0305
	Range	12.1 - 18.7	7.5 - 8.5			
	S.E. of Mean	0.7063	0.1054			
	Coeff. of Variation	13.54%	3.79%			
Female	Mean	15.58	8.00	3.205	0.2434	0.0304
	Range	13.6 - 17.0	7.4 - 8.2			
	S.E. of Mean	0.4534	0.1237			
	Coeff. of Variation	7.13%	3.79%			
Combination	Mean	15.11	7.91	3.203	0.2415	0.0305
	Range	12.1 - 18.7	7.4 - 8.5			
	S.E. of Mean	0.4478	0.0800			
	Coeff. of Variation	11.09%	3.77%			

GROWTH SURVEY — BODY

Litter Ref. EL/120 — Age: 10 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	15.51	7.94	3.193	0.2589	0.0310
	Range	13.5 - 18.4	7.5 - 8.3			
	S.E. of Mean	0.7769	0.1020			
	Coeff. of Variation	13.25%	3.40%			
Female	Mean	16.20	7.93	3.135	0.2576	0.0325
	Range	15.5 - 16.7	7.8 - 8.2			
	S.E. of Mean	0.1789	0.0843			
	Coeff. of Variation	2.70%	2.61%			
Combination	Mean	15.83	7.94	3.166	0.2511	0.0316
	Range	13.5 - 18.4	7.5 - 8.3			
	S.E. of Mean	0.4224	0.0648			
	Coeff. of Variation	9.62%	2.93%			

GROWTH SURVEY — BODY

Litter Ref. EL/102 — Age: 11 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	18.39	8.58	3.259	0.2498	0.0291
	Range	15.3 - 20.8	7.8 - 9.0			
Male	S.E. of Mean	0.4636	0.0959			
	Coeff. of Variation	9.09%	4.03%			
	Mean	17.10	8.40	3.337	0.2423	0.0289
	Range	14.8 - 19.0	8.3 - 8.5			
Female	S.E. of Mean	1.2288	0.0574			
	Coeff. of Variation	12.45%	1.19%			
	Mean	18.15	8.55	3.478	0.2483	0.0290
	Range	14.8 - 20.8	7.8 - 9.0			
Combination	S.E. of Mean	0.4409	0.0800			
	Coeff. of Variation	9.72%	3.75%			

GROWTH SURVEY — BODY

Litter Ref. EL/117 — Age: 12 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	20.95	8.46	3.072	0.2927	0.0346
	Range	18.0 - 22.3	8.3 - 8.6			
Male	S.E. of Mean	0.4946	0.0500			
	Coeff. of Variation	6.68%	1.66%			
	Mean	20.78	8.60	3.127	0.2810	0.0327
	Range	19.2 - 22.0	8.1 - 9.0			
Female	S.E. of Mean	0.5426	0.1761			
	Coeff. of Variation	5.84%	4.58%			
	Mean	20.88	8.52	3.093	0.2876	0.0338
	Range	18.0 - 22.3	8.1 - 9.0			
Combination	S.E. of Mean	0.3551	0.0721			
	Coeff. of Variation	6.13%	3.06%			

Litter Ref. EL/103 Age: 13 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	21.29	8.88	3.210	0.2700	0.0304
	Range	18.5 - 25.5	8.4 - 9.3			
	S.E. of Mean	0.6468	0.1015			
	Coeff. of Variation	10.08%	3.80%			
Female	Mean	19.26	8.90	3.321	0.2432	0.0273
	Range	17.6 - 21.0	8.2 - 9.4			
	S.E. of Mean	0.6882	0.1949			
	Coeff. of Variation	7.99%	4.9%			
Combination	Mean	20.66	8.89	3.245	0.2614	0.0294
	Range	17.6 - 25.5	8.2 - 9.4			
	S.E. of Mean	0.5388	0.0889			
	Coeff. of Variation	10.43%	4.00%			

GROWTH SURVEY — BODY

Litter Ref. EL/110 — Age: 14 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	25.81	9.24	3.130	0.3023	0.0327
	Range	21.7 - 27.6	8.7 - 9.7			
	S.E. of Mean	0.7463	0.1249			
	Coeff. of Variation	7.65%	3.58%			
Female	Mean	24.53	9.10	3.135	0.2962	0.0326
	Range	20.1 - 26.0	8.5 - 9.5			
	S.E. of Mean	0.9025	0.1439			
	Coeff. of Variation	9.01%	3.87%			
Combination	Mean	25.22	9.18	3.132	0.2993	0.0326
	Range	20.1 - 27.6	8.5 - 9.7			
	S.E. of Mean	0.5835	0.0927			
	Coeff. of Variation	8.34%	3.64%			

GROWTH SURVEY — BODY

Litter Ref. EL/105 — Age: 15 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	19.59	8.56	3.183	0.2674	0.0312
	Range	15.0 - 23.5	7.9 - 9.3			
	S.E. of Mean	0.9009	0.1425			
	Coeff. of Variation	14.54%	5.26%			
Female	Mean	19.88	8.90	3.288	0.2510	0.0282
	Range	18.7 - 22.6	8.5 - 9.4			
	S.E. of Mean	0.4328	0.1054			
	Coeff. of Variation	6.16%	3.34%			
Combination	Mean	19.72	8.71	3.230	0.2599	0.0298
	Range	15.0 - 23.5	7.9 - 9.4			
	S.E. of Mean	0.5237	0.0985			
	Coeff. of Variation	11.27%	4.77%			

GROWTH SURVEY BODY

Litter Ref. EL/109 Age: 16 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	27.90	9.67	3.190	0.2984	0.0309
	Range	25.0 - 30.0	9.2 - 10.1			
	S.E. of Mean	0.4397	0.0843			
	Coeff. of Variation	5.23%	2.89%			
Female	Mean	25.00	9.33	3.177	0.2872	0.0308
	Range	22.0 - 28.0	8.9 - 9.6			
	S.E. of Mean	1.2247	0.1493			
	Coeff. of Variation	9.80%	3.20%			
Combination	Mean	27.13	9.58	3.191	0.2956	0.0309
	Range	22.0 - 30.0	8.9 - 10.1			
	S.E. of Mean	0.5517	0.0819			
	Coeff. of Variation	7.88%	3.31%			

GROWTH SURVEY — BODY

Litter Ref. EL/112 — Age: 17 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	28.68	9.76	3.193	0.3011	0.0308
	Range	24.0 - 31.4	9.0 - 10.4			
	S.E. of Mean	0.8656	0.1476			
	Coeff. of Variation	8.54%	4.28%			
Female	Mean	26.80	9.64	3.225	0.2884	0.0299
	Range	24.1 - 30.0	9.2 - 10.0			
	S.E. of Mean	0.8313	0.1233			
	Coeff. of Variation	8.21%	3.38%			
Combination	Mean	27.80	9.71	3.208	0.2949	0.0304
	Range	24.0 - 31.4	9.0 - 10.4			
	S.E. of Mean	0.6329	0.0954			
	Coeff. of Variation	8.82%	3.80%			

Litter Ref. EL/106 Age: 18 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	26.81	9.80	3.278	0.2792	0.0285
	Range	21.7 - 30.2	9.2 - 10.3			
	S.E. of Mean	0.8645	0.1131			
	Coeff. of Variation	9.67%	3.46%			
Female	Mean	26.90	9.73	3.247	0.2841	0.0292
	Range	25.2 - 28.0	9.5 - 10.1			
	S.E. of Mean	0.4115	0.0883			
	Coeff. of Variation	3.75%	2.22%			
Combination	Mean	26.85	9.77	3.266	0.2813	0.0288
	Range	21.7 - 30.2	9.2 - 10.3			
	S.E. of Mean	0.5297	0.0748			
	Coeff. of Variation	7.64%	2.96%			

GROWTH SURVEY BODY

Litter Ref. EL/108 Age: 19 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	27.27	9.90	3.292	0.2782	0.0281
	Range	24.7 - 31.1	9.3 - 10.3			
	S.E. of Mean	1.0349	0.1572			
	Coeff. of Variation	9.30%	3.89%			
Female	Mean	24.88	9.79	3.360	0.2596	0.0265
	Range	21.3 - 30.0	9.2 - 10.5			
	S.E. of Mean	0.9676	0.1597			
	Coeff. of Variation	11.00%	4.61%			
Combination	Mean	25.90	9.84	3.331	0.2675	0.0272
	Range	21.3 - 31.1	9.2 - 10.5			
	S.E. of Mean	0.7566	0.1105			
	Coeff. of Variation	10.93%	4.19%			

GROWTH SURVEY — BODY

Litter Ref. EL/111 — Age: 20 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	30.64	10.64	3.403	0.2706	0.0254
	Range	28.6 - 33.3	10.1 - 11.0			
	S.E. of Mean	0.6222	0.1288			
	Coeff. of Variation	5.37%	3.20%			
Female	Mean	29.57	10.54	3.409	0.2662	0.0253
	Range	27.5 - 32.0	10.0 - 11.0			
	S.E. of Mean	1.1047	0.1360			
	Coeff. of Variation	9.88%	3.41%			
Combination	Mean	30.11	10.59	3.407	0.2685	0.0254
	Range	27.5 - 33.3	10.0 - 11.0			
	S.E. of Mean	0.6269	0.0911			
	Coeff. of Variation	7.79%	3.22%			

GROWTH SURVEY

Litter Ref. EL/104 Age: 21 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
	Mean	31.72	10.2	3.236	0.3049	0.0299
	Range	23.1 - 38.4	9.3 - 11.0			
Male	S.E. of Mean	2.5889	0.2569			
	Coeff. of Variation	19.99%	6.17%			
	Mean	34.21	10.25	3.161	0.3256	0.0326
	Range	28.3 - 39.7	9.4 - 11.0			
Female	S.E. of Mean	1.3171	0.1715			
	Coeff. of Variation	12.18%	5.29%			
	Mean	33.28	10.23	3.189	0.3180	0.0311
	Range	23.1 - 39.7	9.3 - 11.0			
Combination	S.E. of Mean	1.2592	0.1389			
	Coeff. of Variation	15.13%	5.43%			

GROWTH SURVEY — BODY

Litter Ref. EL/113 — Age: 22 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	39.55	10.83	3.182	0.3372	0.0311
	Range	34.7 - 43.2	10.5 - 11.2			
	S.E. of Mean	1.2347	0.1175			
	Coeff. of Variation	7.65%	2.66%			
Female	Mean	37.04	10.79	3.240	0.3181	0.0295
	Range	30.0 - 44.6	10.0 - 11.4			
	S.E. of Mean	1.5891	0.2057			
	Coeff. of Variation	12.87%	5.72%			

GROWTH SURVEY — BODY

Litter Ref. EL/146 — Age: 23 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	41.0	11.37	3.297	0.3171	0.0279
	Range	38.3 - 43.3	11.2 - 11.5			
	S.E. of Mean	0.5790	0.0361			
	Coeff. of Variation	3.74%	0.84%			
Female	Mean	35.35	10.88	3.316	0.2986	0.0274
	Range	31.0 - 39.1	10.3 - 11.2			
	S.E. of Mean	1.0635	0.1175			
	Coeff. of Variation	8.51%	3.06%			

GROWTH SURVEY — BODY

Litter Ref. EL/129 — Age: 24 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	41.00	11.68	3.392	0.3005	0.0257
	Range	33.7 - 46.5	10.3 - 12.6			
	S.E. of Mean	0.9692	0.3987			
	Coeff. of Variation	5.79%	8.36%			
Female	Mean	40.73	11.36	3.304	0.3156	0.0278
	Range	33.6 - 48.6	10.2 - 12.2			
	S.E. of Mean	1.8350	0.2666			
	Coeff. of Variation	12.74%	6.64%			

GROWTH SURVEY — BODY

Litter Ref. EL/147 — Age: 25 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	42.47	11.88	3.407	0.3009	0.0253
	Range	35.3 - 47.8	10.8 - 12.6			
	S.E. of Mean	1.4938	0.2032			
	Coeff. of Variation	10.55%	5.13%			
Female	Mean	40.46	11.63	3.388	0.2991	0.0257
	Range	34.3 - 45.2	10.8 - 12.1			
	S.E. of Mean	1.0694	0.1600			
	Coeff. of Variation	7.48%	3.89%			

GROWTH SURVEY — BODY

Litter Ref. EL/130 — Age: 26 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	48.12	11.96	3.294	0.3364	0.0281
	Range	33.0 - 58.2	10.6 - 12.9			
	S.E. of Mean	2.1598	0.7058			
	Coeff. of Variation	14.19%	4.17%			
Female	Mean	47.64	11.92	3.295	0.3353	0.0281
	Range	38.4 - 55.0	11.2 - 12.4			
	S.E. of Mean	2.6544	0.2332			
	Coeff. of Variation	12.46%	4.38%			

GROWTH SURVEY — BODY

Litter Ref. EL/137 — Age: 27 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	49.48	12.48	3.401	0.3177	0.0255
	Range	46.1 - 55.8	12.2 - 13.0			
	S.E. of Mean	1.6533	0.1497			
	Coeff. of Variation	7.47%	2.68%			
Female	Mean	47.46	11.88	3.282	0.3363	0.0283
	Range	45.1 - 51.8	11.6 - 12.3			
	S.E. of Mean	0.7263	0.0938			
	Coeff. of Variation	4.59%	2.37%			

GROWTH SURVEY — BODY

Litter Ref. EL/131 — Age: 28 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	55.21	12.54	3.298	0.3511	0.0280
	Range	40.8 - 66.3	11.3 - 13.3			
	S.E. of Mean	2.1828	0.1868			
	Coeff. of Variation	13.11%	4.94%			
Female	Mean	47.72	11.86	3.283	0.3393	0.0286
	Range	36.0 - 57.6	10.8 - 12.6			
	S.E. of Mean	4.1737	0.3310			
	Coeff. of Variation	19.55%	6.24%			

GROWTH SURVEY — BODY

Litter Ref. EL/143 — Age: 29 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	69.60	13.73	3.392	0.3692	0.0269
	Range	74.6 - 85.0	13.5 - 14.0			
	S.E. of Mean	12.6116	0.1453			
	Coeff. of Variation	31.39%	1.83%			
Female	Mean	70.28	13.18	3.196	0.4046	0.0307
	Range	66.5 - 74.2	12.0 - 13.6			
	S.E. of Mean	0.6999	0.0922			
	Coeff. of Variation	3.30%	2.32%			

GROWTH SURVEY — BODY

Litter Ref. EL/132 — Age: 30 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	75.47	13.72	3.250	0.4009	0.0323
	Range	69.7 - 81.6	13.4 - 14.2			
	S.E. of Mean	1.6254	0.1304			
	Coeff. of Variation	5.28%	2.32%			
Female	Mean	69.38	12.72	3.094	0.4288	0.0337
	Range	66.4 - 72.9	11.8 - 13.2			
	S.E. of Mean	0.9579	0.2287			
	Coeff. of Variation	3.38%	4.40%			

GROWTH SURVEY — BODY

Litter Ref. EL/144 — Age: 31 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	71.19	13.66	3.297	0.3815	0.0279
	Range	54.6 - 85.0	12.3 - 14.4			
	S.E. of Mean	2.8352	0.2225			
	Coeff. of Variation	11.95%	4.89%			
Female	Mean	57.87	12.77	3.318	0.3536	0.0278
	Range	39.1 - 71.5	11.0 - 13.8			
	S.E. of Mean	5.3457	0.4681			
	Coeff. of Variation	22.63%	8.98%			

GROWTH SURVEY — BODY

Litter Ref. EL/133 — Age: 32 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	58.35	13.83	3.565	0.3051	0.0221
	Range	52.4 - 66.1	13.4 - 14.3			
	S.E. of Mean	1.8401	0.1543			
	Coeff. of Variation	7.72%	2.73%			
Female	Mean	55.41	14.01	3.675	0.2823	0.0201
	Range	49.5 - 62.2	13.1 - 15.5			
	S.E. of Mean	1.7229	0.3412			
	Coeff. of Variation	8.23%	6.44%			

GROWTH SURVEY — BODY

Litter Ref. EL/139 — Age: 33 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	78.97	14.26	3.326	0.3883	0.0272
	Range	67.0 - 88.9	13.4 - 14.7			
	S.E. of Mean	2.4601	0.1673			
	Coeff. of Variation	8.24%	3.11%			
Female	Mean	73.03	13.63	3.270	0.3931	0.0288
	Range	53.3 - 90.2	12.5 - 11.6			
	S.E. of Mean	4.3283	0.2534			
	Coeff. of Variation	16.76%	5.26%			

GROWTH SURVEY — BODY

Litter Ref. EL/134 — Age: 34 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	51.90	13.50	3.617	0.2848	0.0211
	Range	49.0 - 56.1	13.2 - 14.0			
	S.E. of Mean	1.1929	0.1342			
	Coeff. of Variation	5.14%	2.22%			
Female	Mean	48.43	13.36	3.669	0.2713	0.0203
	Range	43.2 - 54.6	12.9 - 14.2			
	S.E. of Mean	1.2935	0.1476			
	Coeff. of Variation	7.55%	3.12%			

GROWTH SURVEY — BODY

Litter Ref. EL/145 — Age: 35 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	81.84	14.26	3.285	0.4025	0.0282
	Range	74.2 - 87.4	13.8 - 14.5			
	S.E. of Mean	1.6535	0.1149			
	Coeff. of Variation	5.71%	2.28%			
Female	Mean	65.38	13.08	3.249	0.3821	0.0292
	Range	48.1 - 75.6	11.2 - 14.2			
	S.E. of Mean	5.9760	0.6651			
	Coeff. of Variation	18.28%	10.17%			

GROWTH SURVEY — BODY

Litter Ref. EL/124 — Age: 36 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	70.31	14.23	3.452	0.3472	0.0244
	Range	59.2 - 86.3	13.7 - 15.4			
	S.E. of Mean	3.5071	0.2466			
	Coeff. of Variation	13.20%	4.59%			
Female	Mean	62.23	13.87	3.504	0.3235	0.0233
	Range	52.4 - 69.0	12.9 - 14.5			
	S.E. of Mean	2.1735	0.1900			
	Coeff. of Variation	9.24%	3.62%			

GROWTH SURVEY — BODY

Litter Ref. EL/140 — Age: 37 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	66.97	13.80	3.399	0.3517	0.0255
	Range	60.7 - 72.0	13.3 - 14.2			
	S.E. of Mean	1.2956	0.0872			
	Coeff. of Variation	6.12%	1.99%			
Female	Mean	63.39	13.47	3.380	0.3494	0.0259
	Range	57.7 - 67.4	13.0 - 13.9			
	S.E. of Mean	1.4574	0.1105			
	Coeff. of Variation	6.08%	2.17%			

GROWTH SURVEY — BODY

Litter Ref. EL/135 — Age: 38 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	84.55	13.86	3.165	0.4401	0.0318
	Range	64.1 - 94.4	12.3 - 14.3			
	S.E. of Mean	3.4828	0.2472			
	Coeff. of Variation	11.65%	5.04%			
Female	Mean	72.98	13.44	3.221	0.4040	0.0301
	Range	55.1 - 84.2	12.0 - 13.9			
	S.E. of Mean	3.1571	0.2236			
	Coeff. of Variation	12.24%	4.70%			

GROWTH SURVEY — BODY

Litter Ref. EL/142 — Age: 39 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	95.18	15.25	3.341	0.4093	0.0268
	Range	83.9 - 105.7	14.7 - 16.0			
	S.E. of Mean	2.8910	0.2093			
	Coeff. of Variation	8.78%	3.36%			
Female	Mean	86.80	14.78	3.340	0.3973	0.0269
	Range	78.3 - 93.8	14.3 - 15.0			
	S.E. of Mean	2.1561	0.1077			
	Coeff. of Variation	6.08%	1.79%			

Litter Ref. EL/136 Age: 40 DAYS

	Analysis	Weight (Grms.)	Head-Body Length (Cms.)	Ponderal Index	Obesity Index	Rohrer Index
Male	Mean	84.33	15.08	3.438	0.3708	0.0246
	Range	78.7 - 91.8	14.4 - 15.7			
	S.E. of Mean	1.5579	0.1459			
	Coeff. of Variation	5.23%	2.74%			
Female	Mean	74.87	14.51	3.445	0.3556	0.0245
	Range	70.7 - 83.3	13.8 - 15.3			
	S.E. of Mean	1.6462	0.1970			
	Coeff. of Variation	5.82%	3.59%			